# ELECTROPHYSIOLOGICAL EFFECTS OF SUBSTRATE MANIPULATION DURING ACUTE MYOCARDIAL ISCHAEMIA

BY

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"The truth is never complete. It is always relative, and research is never finished".

Claude Bernard

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## THE PURPOSE OF THIS THESIS

Over one-third of all deaths in Britain and the United States result from coronary heart disease, the majority of deaths being sudden and relating to the genesis of malignant arrhythmias or ventricular fibrillation during acute myocardial ischaemia. Little is known, however, of the mechanisms of pathogenesis of these arrhythmias and virtually no therapeutic impact has been made on mortality rates.

The initiating event of acute myocardial ischaemia, however, is accompanied not only by gross electrophysiological abnormalities, and impairment of regional blood flow, but also by major disturbances of cardiac metabolism. The resulting "energy crisis" within ischaemic myocardium can be shown to account for many pathological changes of ischaemia in terms of regional contraction, haemodynamics and alterations of the intracellular and extracellular biochemical milieu. Furthermore, there is growing evidence of a metabolic dependency of certain electrophysiological properties of the heart which may be influenced, both directly as a result of energy deprivation, or indirectly as a result of metabolically dependent changes in the biochemical milieu. It follows, therefore, that any measure which enhances the metabolic status of the acutely ischaemic myocardium might be protective, or partially protective, against electrophysiological abnormalities of importance in the pathogenesis of lethal arrhythmias.

This thesis addresses itself, therefore, to the possibility of manipulating the electrophysiological response of the acutely ischaemic myocardium in a metabolic fashion. One approach which, under certain conditions, may be protective against myocardial ischaemic injury is that of a modulation of ischaemic myocardial metabolism by

variation of myocardial substrate availability. More specifically, therefore, this thesis is directed towards an examination of those electrophysiological effects of substrate manipulation of potential benefit during acute ischaemia. Theoretical, experimental and clinical evidence suggests that such beneficial effects could arise, either by an elevation of substrate availability of glucose, or by a reduction of substrate availability of free fatty acids. As such, an examination in man is not possible and as a close relationship is believed to exist between the mechanisms of arrhythmogenesis leading to sudden death in man and those of the early phase of enhanced vulnerability to arrhythmias following experimental coronary occlusion, studies have been designed in such an experimental model over this period of time.

A meaningful examination of the electrophysiological effectiveness of such interventions upon the pathogenesis or mechanisms of pathogenesis of lethal arrhythmias requires, however, an understanding of their pathophysiology. This thesis, therefore, attempts to define the natural history of change of a number of electrophysiological variables, from both intracellular and extracellular recordings, which could relate to early arrhythmogenesis during acute myocardial ischaemia. In addition it attempts to provide evidence concerning the relative importance of re-entrant, as opposed to automatic, mechanisms at this time.

Electrophysiological studies have been designed, therefore, to assess not only the vulnerability of the whole ventricle to development of arrhythmias or ventricular fibrillation, but also more specifically to examine changes likely to enhance the development of re-entrant excitation. In particular, factors predisposing to

re-entrant activity, including inhomogeneity of refractoriness, conduction delays and conduction block, are examined. In addition changes in the transmembrane action potential, which are believed to reflect both alterations in the cellular energy balance and alterations in the intracellular and extracellular milieu, are examined. An assessment of the importance of electrophysiological effects following metabolic intervention in relation to arrhythmogenesis thus is possible.

Much knowledge of cardiac electrophysiological properties and hypotheses of mechanisms of arrhythmogenesis have been derived from studies with isolated tissue. Important effects in vivo during acute ischaemia may result, however, from reflex or neurogenic effects, endocrine influences and haemodynamic changes, such as variations in preload or afterload. For this reason the emphasis in this thesis is upon an examination of electrophysiological effects in the in situ heart and their relation, not only to altered substrate availability, but also to associated alterations in regional myocardial blood flow and metabolic gradients during ischaemia.

The scope of this thesis, therefore, is an examination, both of the electrophysiological changes of acute myocardial ischaemia and their relation to arrhythmogenesis, and of the effects on these changes of metabolic intervention by substrate manipulation, these being examined in the intact heart in relation to alterations in myocardial blood flow and metabolism.

The aim of the experimental work presented in this thesis is both to derive basic knowledge of the inter-relationship between cardiac metabolism and electrophysiology during acute myocardial ischaemia, and to seek some possible means of therapeutic intervention which may be applicable to the prevention of sudden death in man from coronary heart disease.

## THE PLAN OF THIS THESIS

This thesis is divided into three major sections.

An introductory review is given of current knowledge concerning the inter-relationship between changes in cardiac metabolism and electrophysiology during acute myocardial ischaemia. Both the electrophysiological and metabolic changes of acute ischaemia are described, together with known metabolic influences on cardiac electrophysiology. Finally, the literature is reviewed of effects of substrate manipulation during ischaemia, particularly with respect to the "glucose" and "fatty acid" hypotheses.

Secondly, an account is given of the experimental model chosen of the open-chest anaesthetised dog which permits combined measurements of electrophysiological variables, regional myocardial blood flow and metabolic gradients following coronary occlusion. Developmental studies, validation and description of three separate electrophysiological techniques are given for the assessment of a) ventricular vulnerability using a ventricular premature beat threshold b) regional changes in refractoriness c) combined recording of epicardial action potentials and endocardial-epicardial conduction delays. Utilising these experimental techniques, electrophysiological effects, together with alterations in metabolic gradients and regional myocardial blood flow, are presented following acute coronary occlusion with and without the potentially beneficial substrate manipulations of elevation of plasma glucose concentrations and reduction of elevated plasma free fatty acid concentrations.

The final section discusses these experimental findings in terms both of pathological mechanisms and potential therapeutic applications. The main conclusions of this thesis are presented.

ABSTRACT

Alterations of substrate availability may modulate the energy balance within ischaemic myocardium. Electrophysiological effects of substrate manipulation have been examined, therefore, in the open chest anaesthetised dog following acute experimental coronary arterial occlusion over the period of the early phase of enhanced ventricular vulnerability to arrhythmias.

Three experimental models are described, permitting combined measurements of myocardial metabolic gradients (arterial-local venous differences), regional myocardial blood flow (RMBF) and a) ventricular premature beat thresholds b) regional ventricular refractory periods (RP) and c) epicardial action potential (AP) and endocardial-epicardial conduction (CT) changes, both before and during successive short periods of occlusion of the left anterior descending coronary artery.

Coronary occlusion resulted in a phase of enhanced ventricular vulnerability to arrhythmias (lowered VPBT), maximal after five minutes of ischaemia; varied patterns of change of RP, with RP prolongation dominant in central ischaemic zones and RP shortening in border zones; AP shortening, "slow response" type activity, electrical alternans and increasing CT. Ventricular fibrillation (VF) was preceded by maximal RP divergence, maximal CT, AP shortening, alternans and intermittent conduction block. Effects were enhanced by increased heart rate and in initial occlusions.

Elevation of plasma glucose concentrations during moderate ischaemia (c.50% normal RMBF) increased VPBT between two and seven minutes after occlusion, compared with control mannitol infusion; reduced VPB frequency; reduced RP shortening and RP gradients; reduced AP shortening, CT and ST-segment elevation and increased

arterial-local venous differences of glucose without change in RMBF. During severe ischaemia (high proximal occlusion) there was no effect on VF incidence and transient effects only on AP shortening and CT.

Inhibition of isoprenaline stimulated lipolysis during moderate ischaemia resulted in halving of arterial free fatty acid and glycerol concentrations and small reductions in RP gradients, AP shortening, CT and ST-segment elevation, but no change in VPBT. There was no effect on RMBF.

# INTRODUCTION

# 1. INTRODUCTION

Death from ischaemic heart disease is a major problem in the Western World. Furthermore, Scotland is second only to Finland in having the highest incidence in the world for men aged 35 - 44 (W.H.O. Statistics Annual, 1975). Despite an overall reduction in male mortality in the 45 - 49 age group, deaths from heart disease have doubled in the last 20 years (British Medical Journal, 1977), and account for 39% of all male deaths in the 45 - 54 year age group (Fulton et al, 1978).

The majority of such deaths occur before the patient reaches hospital and are often without warning symptoms (Gordon and Kannel, 1971; Gillum et al, 1976; Oliver, 1975). Indeed most deaths occur within the first few minutes after onset of the event (Oliver , 1969). Furthermore, evidence from rapid response cardiopulmonary resuscitation by the Seattle Fire Department (Cobb et al, 1976) suggests that the electrophysiological disturbance precipitating circulatory collapse is that of ventricular fibrillation. This may be promptly reversed by external cardiac massage and application of a defibrillating current without subsequent progression to myocardial infarction. Only 57 of 305 patients promptly resuscitated from out-of-hospital ventricular fibrillation developed infarction (Cobb et al, 1976). Furthermore, there appears to be no definite correlation between the extent of coronary artery disease and onset of ventricular fibrillation (Weaver et al, 1976). Abnormal electrophysiological activity is, therefore, one of many manifestations of acute myocardial ischaemia.

Myocardial ischaemia

The initiating event appears to be that of a partial or complete coronary arterial occlusion, either as a result of atherosclerotic narrowing of the vessel, platelet aggregation on an atheromatous plaque (Genton et al, 1973), haemorrhage into an atheromatous plaque (Ross and Glomset, 1976), secondary thrombosis (Chandler et al, 1974; Fulton et al, 1978) or coronary arterial spasm (Maseri , 1975). This is characterised clinically by angina pectoris, unstable angina, or the atypical angina of Prinzmetal, and may progress to acute myocardial infarction. Simulation in the experimental animal is possible by clip occlusion of a coronary vessel.

Myocardial ischaemia so resulting may be defined as a state of deficiency of coronary perfusion relative to the normal metabolic demands of the myocardium (Maseri, 1975). A situation is created, therefore, whereby oxygen supply to the myocardium is insufficient to meet the oxygen demands of the tissue.

This potentially reversible state should be clearly distinguished from myocardial infarction with irreversible changes of cell death, which may develop after 30 - 40 minutes of severe ischaemia (Jennings and Ganote, 1972).

The degree of ischaemia can be increased not only by a further reduction in coronary perfusion, but also by an increase in oxygen requirement of the heart or by a decrease in the metabolic efficiency of the heart.

Two major therapeutic approaches have been adopted to the management of electrophysiological disturbances during acute myocardial ischaemia. The first is the use of specific anti-arrhythmic

agents, which have been categorised into four major classes (Vaughan Williams, 1970) according to their mode of action as local anaesthetic agents (class 1), sympathetic blockers (class 2), of prolongation of refractoriness (class 3) or calcium antagonism (class 4). Several drugs have more than one class of action. Although of great practical importance, this pharmacological approach will not be considered further in this thesis.

The alternative approach is to improve cellular and hence electrophysiological function by reducing the degree of myocardial ischaemic injury. This may be achieved in four ways:-

a) by decreasing myocardial oxygen requirements: using sympathetic blocking agents (propranolol, practolol), digitalis (in the failing heart), reducing afterload or preload (peripheral vasodilators) or by counterpulsation.

b) by increasing myocardial oxygen supply: by direct coronary revascularisation, by elevating arterial oxygen tension, by use of thrombolytic agents, by enhancing collateral flow by increasing coronary perfusion pressure (methoxamine, noradrenaline), counterpulsation, or hyaluronidase; by increasing plasma osmolality (mannitol).

c) by protecting against autolytic or heterolytic processes: using corticosteroids or cobra venom factor.

d) by substrate manipulation or metabolic intervention: by reducing intracellular free fatty acids using antilipolytic agents, lipid-free albumin solutions or glucose-insulin-potassium solutions, by augmenting glycolytic flux using glucose or glucose-insulin-potassium solutions, or sodium dichloroacetate; by preventing ATP entrapment within the mitochondria using l-carnitine.

It is this latter approach of the possibility of manipulating the electrophysiological response of the acutely ischaemic myocardium by alteration of substrate availability which forms the basis of this thesis.

## 2. ELECTROPHYSIOLOGY OF MYOCARDIAL ISCHAEMIA

### Historical introduction

Awareness of the electrophysiological properties of the heart arose in the last century following the development of the science of electricity and at a time of keen interest in a scientific approach to physiology. Electrical activity from the heart was detected at the body surface in 1887 (Waller) to be followed by the development of the string galvanometer by Einthoven (1913) for recording of the electrocardiogram.

The most important feature of coronary occlusion to early workers - as indeed it is today - was the observation of abnormalities of cardiac rhythm which commonly preceded the terminal event of ventricular fibrillation. Vulpian described this phenomenon as "mouvement fibrillaire" in 1874. It was Porter, however, in 1894 who concluded that fibrillation was a functional disorder rather than an anatomic fragmentation or a break in fibre continuity within the heart. The concept that rhythm disturbances may be initiated by differences in excitability and conduction in different parts of the myocardium was formulated in 1914 by Garrey and subsequently amplified by many workers. Further notable advances were made by Wiggers (1941) who noted that a region of ischaemia was more vulnerable to induction of fibrillation following application of an electrical current and the existence of a "vulnerable period" during diastole; and by Harris (1943) who defined

distinctive phases of abnormal electrical activity and arrhythmias following acute coronary occlusion in the dog.

The impetus of research into basic cardiac electrophysiological mechanisms had to await the development of the micro-electrode technique (Ling and Gerard, 1949). This enabled the clarification of many aspects of normal cardiac electrophysiology (Hoffman, 1960; Noble, 1975), and studies of the simulation of abnormal states by in vitro studies of isolated cardiac tissue (Wit et al, 1974; Cranefeld, 1975). In addition the development of the floating microelectrode technique (Woodbury, 1955) has permitted a direct examination of cellular electrophysiological changes in vivo during ischaemia.

Only in the last ten years have rapid advances been made into our understanding of the pathophysiology of electrophysiological events during myocardial ischaemia and their relationship to associated life-threatening rhythm disturbances.

An understanding of such pathophysiology requires some knowledge of the electrophysiological properties of normal cardiac muscle.

## Electrophysiological properties of the normal heart

Normal cardiac excitation is initiated in the sino-atrial node by specialised pacemaking cells. A wave of excitation passes across the atrium to the atrio-ventricular node which itself possesses pacemaking properties normally suppressed by the more rapid sino-arterial rate. Excitation is then transmitted into bundles of specialised conducting (or Purkinje) muscle cells comprising the bundle of His and left and right bundle branches which allow rapid

transmission of the impulse to both left and right ventricles. Near synchronous activation of ventricular muscle cells is thereby achieved permitting ventricular contraction.

These electrical phenomena can, in large part, be accounted for by changes in passive ionic permeabilities of the cell membrane during excitation. The particular properties of sino-atrial or atrio-ventricular nodal tissue are described by Hoffman (1960) and Noble (1975) and will not be further discussed here.

In the resting state cardiac cells have a transmembrane potential of 80 to 85 mV, the inside being negative with respect to the outside. The level of this potential can be predicted on the basis of the known differential distribution of $Na^+$ and $K^+$ between the intracellular and extracellular spaces and of a selective permeability of the membrane to potassium. So, by the Nernst equation, the membrane potential at which no net $K^+$ current would flow is given by:-

$$E_k = \frac{RT}{ZF} \ln \frac{(K)_o}{(K)_i} \text{ mV}$$

where $E_k$ is the equilibrium potential of $K^+$

R is the perfect gas constant

T is the absolute temperature

Z is the valency of $K^+$

F is the Faraday value

The suffix o denotes outside and i inside the cell

The closeness of the theoretical to the recorded potential under a variety of levels of (K)o would confirm this preferential potassium permeability of the membrane. A reduction in (K)o, however, may induce a hyperpolarisation less than that predicted from the Nernst

equation due to a reduction of potassium permeability (Carmeliet, 1961).

The actual membrane potential (Em) is not exactly $E_k$ as other ions may influence the resting potential according to the Goldman-Hodgkin-Katz equation:-

$$Em = \frac{RT}{F} \ln \frac{PNa\ (Na)i + Pk\ (K)o + Pcl\ (Cl)o}{PNa\ (Na)o + Pk\ (K)i + Pcl\ (Cl)i}$$

where P is permeability and the suffixes o and i refer to extracellular and intracellar respectively.

The ionic concentration gradients necessary for the maintenance of the transmembrane potential are generated by energy-dependent ionic pumping mechanisms (Section 3).

Excitation in cardiac muscle, as in skeletal muscle, involves the reversal in polarity of the transmembrane resting potential, giving rise to the recorded cardiac action potential (Figure 1). The action potential itself consists of two major components, an early phase of rapid depolarisation and a slower plateau phase. Recent studies using micro-electrodes and the voltage clamp technique helped to unravel some of the ionic mechanisms involved. In the same manner as shown in the classical studies of Hodgkin and Huxley for nerve action potential, the various electrical changes of the cardiac action potential can be accounted for by alterations of permeabilities or conductances of a number of specific ionic channels in the cells membrane.

Figure 1 illustrates the various changes in ionic conductances believed to take place during the course of a cardiac action potential. Seven channels are shown and more may exist.

Two major inward currents are believed responsible for the depolarisation phase of the cardiac action potential; a rapid inward

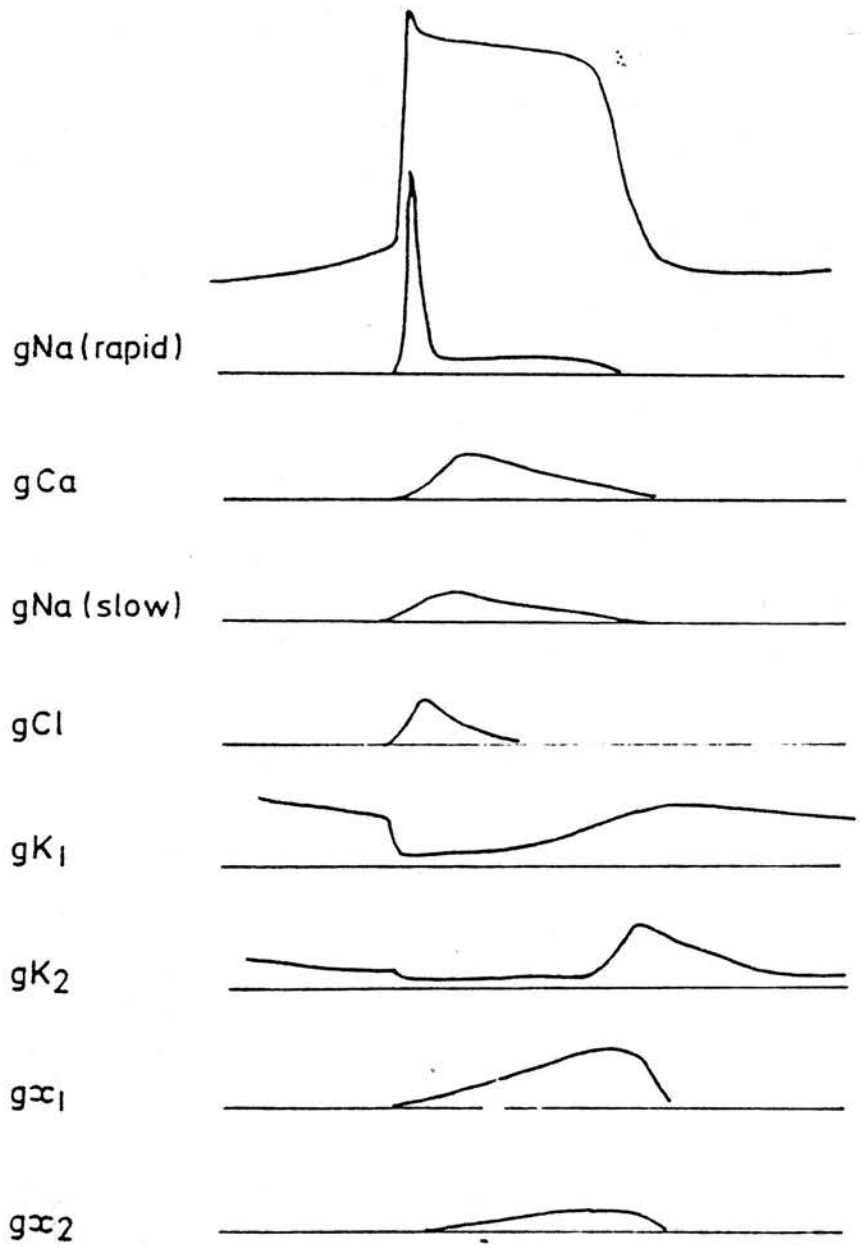


FIG. 1. Diagrammatic illustration of ionic conductances underlying electrical activity in the heart

(after Coraboeuf et al, 1976)

current carried by sodium ions determined by a sudden increase in sodium conductance ($g_{Na}$) which results in the initial rapid depolarisation; and a slower inward current carried largely by calcium ions, but also some $Na^+$ ions which results in the characteristic plateau. Conductance characteristics of sodium ($g_{Na}$) and calcium ions ($g_{Ca}$) are each in turn dependent upon activation (m and d) and inactivation (h and f) variables (Noble, 1975). Thus the sodium current, $i_{Na}$, and the calcium current, $i_{Ca}$, are given by:-

$$i_{Na} = m^3 h \bar{g}_{Na} (Em - E_{Na})$$

$$i_{Ca} = d^n f g_{Ca} (Em - E_{Ca})$$

where $\bar{g}_{Na}$ and $\bar{g}_{Ca}$ refer to ionic conductances when the postulated gating channel is fully open. The value of n is not yet defined. Maintenance of the 'plateau' phase of the action potential is achieved by a balance of several ionic currents. A decrease in outward $K^+$ current and $Cl^-$ currents offset a simultaneous decrease in slow inward current carried primarily by calcium ions. In addition anomalous rectification of potassium occurs.

An important property of the sodium channel is that $g_{Na}$ is completely inactivated by a depolarisation of 30 - 40 mV, whereas $g_{Ca}$ is only inactivated by depolarisation of 80 mV or more. Thus when the membrane is partially depolarised, as from an accumulation of extracellular potassium, a slow active depolarisation or "slow response" potential can arise. Such potentials can arise in both Purkinje tissue and ventricular muscle cells (Cranefeld, 1975) and are not dissimilar in appearance to the potentials observed in pacemaking cells. The importance of the slow potential lies in its slow

conduction characteristics as velocity of impulse propogation, $\theta$, is related to the velocity of initial membrane depolarisation dV/dt:-

$$\text{viz. } \theta = \frac{r \, d^2V/dt^2}{2 \, Ri \, (Cm.\frac{dV}{dt} + Im)}$$

where r = fibre radius
V = membrane voltage
Cm = specific membrane capacitance
Ri = resistance
Im = membrane current density

The difference between "slow response" and normal "fast response" potentials are described in Figure 2 and Table 1 (adapted from Zipes et al, 1975).

TABLE 1 Comparison of properties of slow and fast response potentials

| Properties | Fast response | Slow response |
|---|---|---|
| Kinetics | rapid | slow |
| Dependent on extracellular concentration of | $Na^+$ | $Ca^{++}$ |
| Abolished by | Tetrodotoxin | Mn, Ca, Ni, La, verapamil |
| Resting potential | -80 to -95 mV | -40 to -70 mV |
| Conduction velocity | 0.5 to 3.0 $m.sec^{-1}$ | 0.01 to 0.1 $m.sec^{-1}$ |
| dV/dt | 200 - 1000 $V.sec^{-1}$ | 1 - 10 $V.sec^{-1}$ |
| response to stimulus | all - or - none | variable |
| recovery of excitability | prompt | delayed, outlasts full repolarisation |

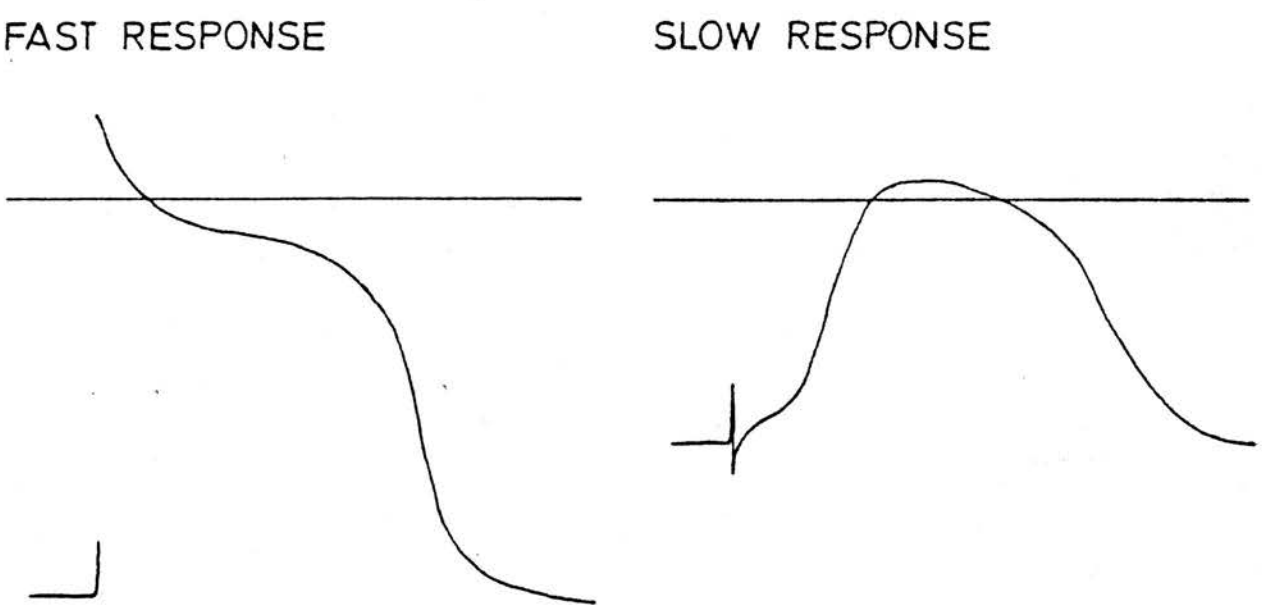


FIG. 2. Comparison of slow and fast response transmembrane cardiac action potentials

(after Wit et al, 1974)

Repolarisation is initiated when outward currents (influx of $Cl^-$ or net efflux of $K^+$) exceed inward currents. The notch (phase 1) on the spike of the action potential is thought to be due to $Cl^-$ flux. Later repolarisation results from a balance between increasing outward current (delayed rectification of $K^+$) and diminishing slow inward calcium current. Two distinct components, $x_1$ and $x_2$, of outward repolarising current have been described (Noble and Tsien, 1969; Brown and Noble, 1969) and are probably regulated by changes in internal calcium concentration (Isenberg, 1974).

The presence of a prolonged plateau phase confers on cardiac tissue the property of greatly prolonged refractoriness compared with other excitable tissues. Teleologically, this may be regarded as a protective adaptation. The refractory period is the period during which the cardiac cell cannot produce a propagated response following any stimulus. Abnormalities in refractoriness will be seen to play a role in the genesis of arrhythmias.

A further fundamental property of cardiac tissue is that of automaticity, seen in specialised conducting tissue, but not under normal conditions in ventricular muscle. Delayed diastolic depolarisation (see Figure 1) progresses until a threshold potential is attained which leads to propagation of a further cardiac action potential. It is believed to result from a time-dependent decline in outward potassium current ($i_{K2}$) (Noble and Tsien, 1968). A second type of automaticity similar to that of sino-atrial node tissue can occur in partially depolarised Purkinje tissue showing slow response activity (Carmeliet and Vereecke, 1969).

## Electrophysiology of ischaemia

Complex electrophysiological changes occur during acute myocardial ischaemia which may result in cardiac arrhythmias or ventricular fibrillation.

Over 50% of deaths associated with coronary artery disease in man occur within the first two hours of onset of acute ischaemia (Armstrong et al, 1972) and appear to result primarily from ventricular fibrillation (Geddes et al, 1967; Adgey et al, 1971). An experimental corollary in the dog has been shown by the early studies of Harris (1950). Using a two stage ligation of the left anterior descending coronary artery of the dog to induce arrhythmias and experimental myocardial infarction, distinct phases of arrhythmias can be demonstrated. An early phase was observed almost immediately after coronary occlusion which lasted for a few hours; after a period of quiescence a second later phase of arrhythmias began at six to nine hours and persisted for 24 - 48 hours. It seems probable that similar early and late phases of arrhythmia production occur in man (Bigger et al, 1977). Furthermore, it is believed that quite different electrophysiological abnormalities and mechanisms of pathogenesis of arrhythmias are operative in these two phases.

In view of their greater clinical importance, emphasis will be placed in this thesis upon electrophysiological changes occurring acutely after coronary occlusion over the period of induction of early (or Harris phase 1) arrhythmias.

It has been known since the work of Pardee (1920) that a consistent electrophysiological abnormality following acute experimental coronary occlusion is an elevation of the ST-segment of the electrocardiogram. Various studies have been performed in an

attempt to elucidate the underlying electrophysiological mechanisms of this change, yet knowledge is still incomplete. Theoretically, ST-segment elevation could arise in one or two ways; either by a current of injury induced by relative depolarisation of ischaemic with respect to normal cells, so producing a current flow in diastole and a baseline DC shift in the TQ segment of the electrocardiogram; or by current flow in systole related to irregularities of repolarisation, producing a true ST-segment elevation above the iso-electric line. Combined recording of intracellular and extracellular potentials in the dog (Prinzmetal et al, 1962; Samson and Scher, 1960) confirmed the findings of Nahum et al (1945) of a DC shift in the extracellular electrogram in diastole and demonstrated a loss of action potential amplitude and reduction in transmembrane potential in the ischaemic zone. True ST-segment elevation, as a result of differential action potential shortening in the ischaemic area, was also observed, however.

Samson and Scher (1960) noted a shortening of action potential duration some 40 seconds after coronary artery occlusion producing a primary ST-segment change on the electrogram followed 80 seconds later by a decrease in resting membrane potential producing an injury current in diastole and hence a secondary ST-segment elevation. Kleber et al (1978) noted, by contrast, in the perfused pig heart that the initial event was a loss of membrane potential and TQ segment depression, to be followed later by a combination of TQ depression and ST-segment elevation, attributable to action potential shortening or conduction delays in the ischaemic zone. Similar qualitative changes are described in perfused rabbit heart (Wittig and Vaughan Williams, 1976). Magneto-cardiography studies (Cohen and Kaufman, 1974) suggest, on the other hand, that ST-segment

shifts are entirely related to a DC shift which can be detected within 20 seconds of coronary occlusion. A quantitative analysis or interpretation of ST-segment changes is complicated by morphological factors. Thus a reduction in ST-segment elevation may accompany an increase in the ischaemic zone of myocardium as the angle subtending the border area between normal and ischaemic myocardium from the point of electrode contact is diminished (Holland and Brooks, 1975). Similarly, gross abnormalities of conduction in the ischaemic zone distort the recorded ST-segment elevation due to greatly delayed depolarisation (Maroko and Braunwald, 1972).

In parallel with increasing ST-segment elevation, recordings of the surface bipolar electrogram show a decline in amplitude and increase in duration related to a delay in activation of ischaemic myocardium. Fragmentation of the ischaemic electrogram has been observed (Boineau and Cox, 1973) with prolonged irregular low amplitude activity extending well into diastole, suggesting marked and variable conduction delays within ischaemic tissue. These delays can be recorded as differences between endocardial and epicardial activation in an ischaemic area (Durrer et al, 1971; Cox et al, 1973). The association between such fragmented surface activation and appearance of premature beats or ventricular tachycardia or ventricular fibrillation has been observed (Waldo and Kaiser, 1973; Boineau and Cox, 1973; Scherlag et al, 1974). From a comparison of the electrograms of subendocardial, subepicardial and intramural layers of dog heart, Scherlag et al (1974) concluded that the greatest delays occur in the superficial subepicardial layer, although such delays in activation could also reflect delays in deeper tissue layers.

A plausible explanation of this delayed fragmented electrical activity is that of slowed conduction due to slow response action potentials or markedly depressed fast response action potentials (Cranefield et al, 1973). Such potentials would be expected to arise under conditions of partial membrane depolarisation, recorded during ischaemia.

A second explanation for this fragmented electrical activity could be the emergence of multiple automatic firing ectopic foci in depressed ischaemic tissue. Ventricular muscle is capable of automatic firing under certain conditions of depolarisation (Imanishi and Surawicz, 1974). Automatic activity of this type has never been observed experimentally, however, in the early period after coronary occlusion.

A clearer understanding of the electrophysiological abnormalities of ischaemia may be obtained by an analysis of intracellular potential changes in individual myocardial cells. Loss of membrane potential and action potential amplitude and action potential shortening occur in vitro in isolated papillary muscle (Trautwein et al, 1954) rendered anoxic. That similar changes occur during ischaemia has been confirmed in studies relating to the pathogenesis of ST-segment elevation (vide supra) and in more specific studies in the dog (Czarnecka et al, 1973) and perfused pig heart (Downar et al, 1977) using the floating microelectrode technique. In the Langendorff blood perfused pig heart preparation (Downar et al, 1977) intracellular subepicardial recordings demonstrated a fall in resting potential associated with action potential shortening, a reduction in action potential amplitude and diminished upstroke velocity in the ischaemic area following coronary occlusion. At the same time, delay of activation was

associated with the appearance of the depressed potentials which resemble the so-called "slow response" potentials in morphology. Total unresponsiveness occurred in some cells after 12 to 15 minutes of occlusion, yet near normal action potential configuration could be restored on flushing the occluded coronary artery with saline as late as 40 minutes after occlusion. Action potentials of slow initial upstroke velocity showed alternation of amplitude and duration in association with alternation of repolarisation characteristics of the local extracellular electrocardiogram. Lengthening of local refractoriness followed an initial shortening and originated local 2:1 responses before complete inexcitability. Monophasic action potential recordings, or recordings of injury potential during ischaemia similarly have revealed shortening of action potential duration, decreased rise time and loss of potential amplitude following coronary occlusion in the dog (Kupersmith et al, 1977; Lab, 1978). This has been confirmed in the perfused human heart by microelectrode recordings (van Dam - personal communication).

A further important change following acute coronary occlusion occurs in the property of ventricular refractoriness. Early work by Han (1964) demonstrated inhomogeneities of ventricular refractoriness following acute coronary occlusion between closely adjacent sites of stimulation. Thus a shortening in refractory period was observed at one point and a lengthening of another point a few millimetres distant during ischaemia. This dispersion of refractoriness was increased by sympathetic stimulation and related to a reduced theshold to electrically induced ventricular fibrillation (Han et al, 1966). The natural history of change of refractoriness during ischaemia is undefined with conflicting reports of directional change in

refractoriness following coronary occlusion (Reynolds et al, 1960; Tsuchida, 1965; Han, 1969). Other reports suggest a shortening of refractoriness in early ischaemia, but a lengthening in later ischaemia (Tsuchida, 1965; Elharrar et al, 1977). Shortening of action potential duration should be associated with shortening in refractory period in the absence of alterations in excitability (Hoffman, 1960).

Abnormalities of diastolic excitability threshold are reported, however, during ischaemia (Elharrar et al, 1977). An initial reduction followed by a progressive rise in excitability threshold is demonstrable after coronary occlusion in the dog using an elegant threshold tracking system. Changes are greater in more severe ischaemia. A superimposition of this elevation of threshold is thought to explain the divergence of action potential duration and refractory period (Lazzara, 1978), and emergence of so-called post-repolarisation refractoriness (Lazzara et al, 1975).

A consideration of the metabolic and ionic mechanisms responsible for these effects is largely speculative and is discussed in Section 3.

Changes in specialised conducting tissue during ischaemia are not, in general, believed to be of great importance in the acute phase of ischaemia (Bigger, 1977). Purkinje fibre electrograms recorded from the ischaemic area were not altered during the first eight to twelve hours after experimental occlusion (Cox et al, 1973). A striking resistance of Purkinje tissue to hypoxia or ischaemia is described (Bagdonas et al, 1961). Some effects may result, however, as diminution of Purkinje electrograms has been observed within the first few minutes of ischaemia in the dog (Lazzara et al, 1975). It is not until the delayed phase of arrhythmias that striking

alterations in the properties of conducting tissue are observed with enhancement of automatic activity.

Ventricular vulnerability

An alternative approach to determining the individual electrophysiological changes in ischaemic myocardium is to assess the vulnerability of the ventricle as a whole to the development of arrhythmias or fibrillation. Wiggers demonstrated in 1940 that the application of an extra stimulus in the mid-portion of the T wave, which he designated the "vulnerable period", could induce premature beats or fibrillation. This was later shown to coincide with the "dip" in the anodal excitability curve (Brooks et al, 1955). The observation followed that ventricular fibrillation could be initiated in man by so-called R-on-T ventricular premature beats arising in this period (Smirk, 1942). This has not proved a consistent finding, however, (Lie et al, 1975), probably due to a widening and extension into diastole of the period of vulnerability during ischaemia related to marked delays in epicardial activation. The technique of assessment of vulnerability has been adapted by variation in current strength and application of trains rather than single pulses over the terminal T wave for the determination of a ventricular fibrillation threshold (Han, 1969; Burgess et al, 1971; Battle et al, 1974; Garza et al, 1974; Bloor et al, 1975). Although a discharge of one microjoule (μJ) may elicit a propogated diastolic response, a discharge of 40,000 μJ may be required to precipitate ventricular fibrillation (Axelrod et al, 1975).

Vulnerability to fibrillation has been shown to be greatly reduced during ischaemia (Han, 1969; Battle et al, 1974), following an ectopic beat (Han et al, 1964; Han et al, 1966; Kliks et al, 1972;

Verrier et al, 1974) or during sympathetic or hypothalamic stimulation (Verrier et al, 1975), and may be modulated by variations in vagal tone (Lown et al, 1977). Furthermore, vulnerability following coronary occlusion is greatest (fibrillation threshold lowest) between 3 and 7 minutes after coronary occlusion (Battle et al, 1974; Garza et al, 1974), coinciding exactly with the time of onset of the Harris phase 1 arrhythmia (Harris, 1950). Alternatively, the vulnerable period threshold can be reduced by applying repetitive extrasystoles, a technique designated as sequential R/T pulsing (Thompson and Lown, 1972) and several determinations of ventricular vulnerability obtained (see Section 7). Although useful as a quantitative assessment of arrhythmogenicity, determination of ventricular vulnerability sheds little light on cellular pathogenetic mechanisms.

## Arrhythmogenesis

Two major theories of arrhythmogenesis applicable to acute myocardial ischaemia have been proposed. They are those of enhanced automaticity and of re-entry.

## Enhanced automaticity

An abnormality in the normal automatic mechanism of slow diastolic (phase 4) depolarisation can result in abnormal impulse initiation. Automatic activity is a normal property of nodal and Purkinje tissue and is not normally observed in ventricular muscle.

Automatic activity in specialised conducting tissue is present at two levels of membrane potential - at normal potential levels (fast response) and when partially depolarised, showing slow response activity (Carmeliet, 1978). In ventricular muscle partial

depolarisation (to -60 mV) may induce slow response activity and abnormal automaticity (Surawicz and Imanishi, 1974). In fast fibres spontaneous diastolic depolarisation is enhanced if extracellular potassium or calcium are reduced (Hoffman and Cranefield, 1960), or by hypoxia (Trautwein, 1954). Abnormal automaticity in slow response fibres, however, probably involves different ionic mechanisms with involvement of slow inward current, slow channel repolarisation current, $i_{x2}$, and possibly background inward current (Wit and Bigger, 1975). It is characterised by oscillatory diastolic potentials which increase in amplitude until threshold is attained and a propagated response induced. Alternatively, systolic oscillations have been observed at or near the plateau phase of the action potential (Bigger and Weld, 1975).

Characteristically, normal automatic activity is suppressed by high rates of electrical stimulation, whereas abnormal automaticity is exacerbated (Wit et al, 1974).

In vivo multiple influences may enhance automaticity. Cathecholamines, released during ischaemia, are known to enhance diastolic depolarisation of both fast and slow fibres (Wit et al, 1974). Structural cellular damage has been associated with automatic activity in ventricular intramural regions of acute myocardial infarction (Sølberg et al, 1972) and subendocardial Purkinje tissue (Friedman et al, 1973). In addition the current flow of injury between normal and ischaemic tissue could increase diastolic depolarisation and initiate ectopic impulses (Kleber et al, 1978). It is not known at present whether such phenomena occur in ventricular myocardial cells during the early phase of acute ischaemia.

Re-entry

The concept of re-entry was suggested as early as 1928 by Schmitt and Erlanger and has been clearly demonstrated by many workers. Indeed Sir Thomas Lewis (1925) suggested that a circus movement of the excitation wave might be the cause of ventricular fibrillation.

It is postulated that re-entrant excitation should occur when a cardiac impulse conducts slowly around a depressed area of undirectional conduction block to excite tissue distal to the blocked site, and then re-excite tissue again proximally (Moe, 1975). This can only occur if the impulse within the re-entrant circuit is delayed at least beyond the refractory period of the tissue at its point of origin. The likelihood of re-entry is, therefore, enhanced by a lengthy re-entry pathway, by slowing of conduction velocity and by shortening of refractoriness. Inhomogeneities of refractoriness between adjacent cells may create focal areas of slowing of conduction or unidirectional conduction block (Allesie et al, 1976) may further potentiate this process.

For successful re-entry:

$$\frac{1}{\theta} > t_{RP}$$

where 1 is the circuit path

$\theta$ is the conduction velocity

and $t_{RP}$ the refractory period (Bigger, 1973)

A number of theoretical mechanisms which could be operative during acute ischaemia have been proposed. Moe et al (1964) showed in a computer model that the re-entry circuit may occur at random and need not persist as a stable circuit. Circuits have

been proposed within ventricular muscle, within conducting tissue, between conducting tissue and ventricular muscle due to abnormalities in the so-called gating mechanism of Myerburg (1970) and as a result of regional differences in refractoriness (Wit et al, 1974).

Evidence in favour of re-entrant mechanisms operative during acute myocardial ischaemia is mounting. The major prerequisite of slowed conduction is clearly demonstrated (Durrer et al, 1971; Boineau and Cox, 1973; Scherlag et al, 1974) and its occurrence coincides with the onset of ventricular arrhythmias (El-Sherif, 1975). This slowed conduction can be mediated by slow response or depressed fast response activity in partially depolarised tissue (Cranefield et al, 1973). Secondly, unidirectional conduction block is possible as a result of post-repolarisation refractoriness (Lazzara et al, 1978), inhomogeneity of refractoriness (Allesie et al, 1976), inhomogeneity of excitability threshold (Elharrar et al, 1977) and localised alternation of response (Downar et al, 1977). Thirdly, shortened refractory periods are found at least in early or mild ischaemia (Han, 1972; Levites et al, 1975). Firmly supportive evidence for re-entry is the demonstration of diastolic "bridging" depolarising activity between a normal and ectopic impulse, implying continuity of excitation. Such activity has been demonstrated using composite bipolar electrodes at least in the late myocardial infarction period in the dog (El-Sherif et al, 1976).

Ventricular fibrillation

Ventricular fibrillation may be defined as chaotic asynchronous fractionated activity of the heart (Moe and Abildskov, 1959). Electrocardiographically electrical activity is grossly disorganised.

No clear pattern of re-excitation is evident on the vector loop (Smirk, 1964). Similarly, intracellular recording has shed little light on mechanisms of pathogenesis. Each fibre in the fibrillating heart behaves in the same manner as in the non-fibrillating heart (Surawicz et al, 1967), although activation during repolarisation may result in potentials of decreased upstroke velocity and small potentials which may be local, graded or oscillatory (Czarnecka et al, 1973). Incomplete repolarisation permits rates of stimulation of up to 500 to 700 per minute. Partial synchronisation may occur in two fibres in close proximity, but activity is asynchronous in fibres separated by a distance of 10 mm (Hogencamp et al, 1959). The size of independently functioning units in ventricular fibrillation is, therefore, small. A critical myocardial mass is, however, necessary to maintain functional activity. Atrial fibrillation, for example, cannot be maintained in less than 1 g of tissue (Moe and Abildskov, 1959).

Two major theories have been proposed to account for the initiation of ventricular fibrillation (Surawicz, 1971):-

a) Rapid formation of impulses from single or multiple foci by enhancement of local automaticity.

b) Repetitive disorganised re-entrant excitation.

More complex effects have been suggested, however. Thus re-entrant activity may be initiated by a critically timed extra-stimulus arising from a focus of enhanced automaticity. The concept of the "vulnerable period" has been discussed. Alternatively, the process of "focal re-excitation" may operate (Han, 1972; Kleber, 1978), whereby the ischaemic current of injury may attain such a level as to exceed threshold in repolarised areas. In the presence of inhomogeneity of repolarisation multiple re-entry

circuits could be simultaneously initiated. Specific factors leading to the initiation of ventricular fibrillation, rather than re-entrant ventricular premature beats, still remain largely undefined.

Conclusions

Normal cardiac electrical activity can be accounted for, in large part, by changes in passive ionic permeabilities of the cell membrane during excitation and passage of ions through specific membrane "channels". At diminished resting potentials "slow response" potentials largely dependent upon slow inward calcium current replace the normal "fast response" action potential. Acute myocardial ischaemia results in a number of electrophysiological abnormalities in the in situ heart, including ST-segment elevation, slowing of conduction, loss of membrane potential and reduction of action potential amplitude and duration, inhomogeneity of refractoriness and abnormalities of diastolic excitability threshold. The vulnerability of the ventricle to development of arrhythmias following application of extrastimulus is enhanced. Early arrhythmogenesis following experimental coronary occlusion is more likely related to re-entrant excitation with involvement of slowly conducting "slow response" activity than to enhancement of automaticity. The pathogenesis of ventricular fibrillation may involve additional effects, such as focal re-excitation.

## 3. METABOLIC INFLUENCES ON CARDIAC ELECTROPHYSIOLOGY

Gross metabolic alterations occur in ischaemic tissue following coronary artery occlusion, both in terms of altered intracellular metabolic processes and altered extracellular milieu. An account of

the metabolic dependency of electrophysiological processes, both within normal and ischaemic tissue, is therefore pertinent.

Normal myocardium

Evidence is accumulating that electrical phenomena within the normal cardiac cell are not entirely explicable on the basis of alterations in passive ionic permeabilities.

Active transport mechanisms determine the distribution of ions across the cell membrane. The ionic gradient of potassium across the membrane is maintained by a pumping system, the $Na^+$ $K^+$ -ATP 'ase, (Schwartz et al, 1975). Sodium is pumped out of the cell and potassium into the cell in a ratio of 3:2 linked to the hydrolysis of ATP. The pumping rate is stimulated by low extracellular potassium concentrations or increased intracellular sodium concentrations. In view of the imbalance between sodium extruded and potassium taken up this active transport mechanism is electrogenic (Thomas, 1972). An excess of positive charge is extruded and outward repolarising current generated which leads to a small degree of hyperpolarisation (Vassalle, 1971; Isenberg and Trautwein, 1974).

This electrogenic $Na^+$ pump is thought to account for a number of phenomena. It explains the drop in membrane potential in Purkinje fibres during rapid cooling and hyperpolarisation beyond the equilibrium potential for potassium ($E_k$) on rewarming (Thomas, 1972). It explains overdrive suppression after rapid pacing in Purkinje fibres as the phenomenon is blocked by dinitrophenol, a metabolic inhibitor (Godfraind, 1977). It also accounts for the maintenance of a high resting potential after prolonged exposure of ventricular muscle to oxygen-free solution in vitro (McDonald and McLeod, 1973).

Experimental work largely involves the use of metabolic inhibitors or ouabain which selectively blocks the external $K^+$ site of the $Na^+$ $K^+$ pump. Administration of ouabain causes a small depolarisation in normal tissue. Adrenaline, by contrast, stimulates the $Na^+$ $K^+$ pump (Vasalle, 1971), which may account for its hyperpolarising effect on Purkinje fibres. The above phenomena could be explained in part on the basis of an active inward $K^+$ transport (Carmeliet, 1978). It is possible then that the $Na^+$ pump acts either as a coupled mechanism or an electrogenic mechanism.

Active transport mechanisms also control the levels and compartmentalisation of $Ca^{++}$ within the cell (Baker, 1972). Cytoplasmic $Ca^{++}$ levels are kept low by active uptake into mitochondria and sarcoplasmic reticulum ($Mg^{++}$ $CA^{++}$ ATP 'ase). To maintain the slow inward calcium current during the action potential a $CA^{++}$ extrusion mechanism is operative indirectly linked to metabolism. A $Na^+$ -linked carrier system has been described with a greater affinity of the carrier for $Ca^{++}$ on the inner cell membrane and a greater affinity for $Na^+$ than $Ca^{++}$ on the outer membrane (Reuter and Seitz, 1968). Metabolic energy is required, therefore, for active transport of $Na^+$ by the $Na^+$ pump.

Voltage clamp studies have shown a striking reduction in the slow inward calcium current following metabolic inhibition by cyanide or dinitrophenol (Kohlhardt and Kubler, 1975). In addition to inhibition of active transport mechanisms it has been postulated that there may be an additional metabolic effect on the conductance characteristics of the calcium channel itself ($g_{Ca}$) (Reuter and Scholz, 1977; Schneider and Sperelakis, 1975). Further evidence from studies

of the effect of glucose on the cardiac action potential during anoxia (see Section 5) suggest that this metabolic dependency is related to ATP derived from anaerobic glycolysis.

Alternatively, the theory has been proposed that the number of slow current channels in the membrane may be controlled by cyclic AMP levels in the inner cell membrane by phosphorylation of specific sites in the cell membrane. Catecholamines which enhance cyclic AMP levels could then effect the known increase in slow inward current by an increase in $g_{Ca}$ by an increase in the number of available channels. Metabolic inhibition would operate in reverse fashion.

Evidence for a metabolic influence on the potassium currents of repolarisation in normal tissue remains tenuous. However, in so far as the voltage-time properties of each channel is interrelated, the outward potassium currents cannot be regarded as entirely passive (Carmeliet, 1978).

Ischaemic myocardium

A complex variety of metabolic and biochemical changes follow acute experimental coronary occlusion and are described in greater detail in Section 4.

As a result of deprivation of blood and hence oxygen supply tissue becomes hypoxic or anoxic. Metabolic energy in the form of ATP diminishes rapidly, lactate accumulates, pH falls and potassium, phosphate and other metabolites leak into the extracellular space. At the same time, noradrenaline is released locally from sympathetic nerve terminals within the myocardium, either directly or reflexly and plasma levels of adrenal catechols rise. Metabolic adjustment to altered systemic substrate levels follows (see Section 4).

All of these complex factors interrelate in determining the electrophysiological response of the myocardium to ischaemia.

Certain passive electrophysiological changes might be predicted on the basis of ionic shifts; an accumulation of extracellular potassium should induce membrane depolarisation and hence reduction of dV/dt and of conduction velocity; a decrease in intracellular pH should increase excitability and automaticity and shorten action potential duration (Hecht and Hutter, 1965), whereas a decrease in extracellular pH should have the opposite effect of increasing the potential gradient across the membrane, producing hyperpolarisation, decreased excitability and decreased automaticity. Experimental values do not, however, conform accurately to theoretical predictions on account of metabolically dependent effects.

Both hypoxia (Trautwein et al, 1954) and metabolic inhibition in isolated tissue are associated with loss of resting membrane potential and decrease in amplitude and duration of the action potential plateau, although these changes occur less rapidly than during ischaemia. It is notable that Purkinje tissue is much less susceptible to anoxia than ventricular myocardial cells (Bagdonas et al, 1961).

Loss in resting potential is related to interstitial accumulation of extracellular potassium and a fall in intracellular potassium, but not nearly to the same extent during ischaemia, as predicted from the Nernst equation. A stimulation of the electrogenic sodium pump diminishes the expected fall in membrane potential from $E_k$.

The studies of McDonald et al (1973) indicate that the energy for this stimulated pump may come from the anaerobic production of ATP

from glycolysis. It is possible that the transmembrane potential may be maintained near normal by this mechanism during the early period of ischaemia, even in the presence of a considerable loss of the potassium transmembrane gradient. This situation may be further complicated by secondary effects of other ions. Passive leakage of potassium from the cell is enhanced by a rise in intracellular calcium, but opposed by a fall in pH (Poole-Wilson and Langer, 1975). A fall in pH, however, also inhibits sodium pump activity (Schwartz et al, 1975).

In ischaemia the elevation of extracellular potassium can account for many of the observed changes. Early changes in excitability (Elharrar et al, 1977) and refractoriness can be simulated by injection of potassium. An early phase of arrhythmias can be mimicked by intracoronary potassium injection (Harris et al, 1954). The immediate post-occlusion arrhythmias have been correlated with elevations in coronary venous potassium concentrations (Harris et al, 1954).

Many effects cannot be so easily ascribed to potassium alone. Conduction velocity is depressed as the upstroke velocity (dV/dt) of the action potential is depressed, for example, by potassium depolarisation. During anoxia, however, dV/dt is depressed even if the resting potential is unaltered (Vleugels and Carmeliet, 1975). Recovery from inactivation of the sodium channel appears depressed during anoxia or ischaemic and could account for the elevations of threshold and post-repolarisation refractoriness observed during ischaemia.

The shortening of the plateau of the action potential occurs after only one or two minutes of ischaemia or hypoxia or substrate

depletion (Trautwein et al, 1954; Downar et al, 1977). Changes in pH or extracellular potassium may play a role. An increase in extracellular potassium may induce action potential shortening (Weidmann, 1956). Extracellular acidosis increases and extracellular alkalosis decreases action potential duration (Hecht and Hutter, 1965). During early ischaemia a fall in intracellular pH concomitant with lactate production precedes the decline in extracellular pH and might be expected to induce action potential shortening. It would, however, appear that a reduction in the metabolically dependent slow inward current carried largely by calcium ions is of more importance. Calcium-mediated slow potentials can be reproduced by field stimulation in the presence of isoprenaline in a high potassium medium (Schneider and Sperelakis, 1975) and are rapidly blocked by metabolic inhibition with cyanide or dinitrophenol or hypoxia. In addition, a fall in action potential amplitude and reduction in plateau duration, attributable to reduction of slow inward current, is associated with a fall in glycolytic rate and ATP content (McDonald and Macleod, 1973; Cheneval et al, 1972). Metabolic influences and effects of substrate availability on ischaemic ATP production and its secondary electrophysiological effects are discussed in Section 5.

Electrophysiological effects are further modulated by release of lactate from ischaemic tissue within a few minutes of coronary occlusions. High tissue levels may be rapidly attained. In vitro studies show that lactate shortens the action potential and decreases the amplitude and rate of rise of the action potential (Wissner, 1974). Automatic firing in Purkinje tissue may be increased or decreased (Wissner, 1974). Lactate induced

catecholamine release from nerve terminals may be arrhythmogenic (Wildenthal et al, 1969). High concentrations of lactate block slow response activity by elevation of diastolic excitability threshold (Wojtczak, 1978).

Cathecholamines are well known to be arrhythmogenic. An increase in circulating adrenaline and noradrenaline follows coronary ligation and coincides with the onset of ventricular arrhythmias (Staszewska-Barczak and Ceremuzynski, 1968, 1969). Sympathetic stimulation and catecholamine infusion reduce the ventricular fibrillation threshold (Han et al, 1964; Kliks et al, 1972). There is evidence for noradrenaline release during myocardial ischaemia and in particular on coronary reperfusion (Shahab et al, 1972) from nerve terminals or noradrenaline storage vesicles in the myocardium. The antiarrhythmic effect of sympathectomy is well known (Cox and Robertson, 1936). A distinction should be made between electrophysiological effects of catecholamine release arising indirectly as a result of stimulation of metabolism and enhanced oxygen consumption which may increase the severity of ischaemia, and direct cellular effects. Cellular effects include slight shortening in action potential duration and refractory period, hyperpolarisation of partially depolarised fibres and stimulation of slow response activity in previously inexcitable tissue (Wit et al, 1972). Re-entrant circuits may be induced by enhanced slow conduction in depressed tissue. Enhancement of automatic activity may result in Purkinje tissue or even in ventricular myocardial cells (Wit et al, 1974). Inhomogeneity of effect may also be of importance. A biphasic effect on dispersion of refractoriness and fibrillation threshold is decribed (Han et al, 1964), an initial

increase in dispersion following isoprenaline infusion is followed by a later decrease and rise of fibrillation threshold, thought to be related to initial inhomogeneity, but later homogeneity of perfusion. Marked inhomogeneity of noradrenaline release is likely during acute ischaemia.

Release of unidentified substances from ischaemic tissue may contribute to the electrophysiological abnormalities of acute ischaemia. Downar (1977) demonstrated that ischaemic venous effluent blood had marked depressant effects on myocardial cells in vitro which were not explicable on the basis of alterations in potassium, lactate, pH, glucose, pO2 or free fatty acid concentrations. It was concluded that some ischaemic "depressant factor" may be released following coronary occlusion.

The possibility that release of lysophospholipid (lysophotphatidyl ethanolamine or lysophosphatidylserine) may have some such effect has been examined by Corr et al (1978) following the finding that elevated tissue levels of lysophospholipid are found during myocardial ischaemia. Lysophospholipid at similar concentrations had depressant effects on Purkinje fibre transmembrane potential and membrane responsiveness which were reversible. During the acute phase of ischaemia, however, lysophospholipid is not elevated in tissue biopsies or in local venous effluent blood from acutely ischaemic myocardium in the dog (Mikarowsky - unpublished, 1978).

An additional effect of metabolic inhibition or hypoxia is the promotion of accumulation of calcium in the intercellular nexus and promotion of an electrical uncoupling of adjacent cells. This is manifest by an increase in internal longitudinal resistance (Wojtczak, 1976). The effect is enhanced by catecholamines and may play a role in the initiation of localised conduction blocks in the ischaemic zone.

Alterations in cardiac metabolism therefore modulate the electrophysiological response to ischaemia and are an important determinant of arrhythmogenesis.

Conclusions

Changes in passive ionic permeabilities cannot account for all electrical phenomena in the normal cardiac cell. Ionic gradients are maintained by $Na^+$-$K^+$ and $Na^+$ -$Ca^{++}$ pumps linked to hydrolysis of ATP. Hyperpolarisation can result from the electrogenic $Na^+$ pump. A striking metabolic dependency of the slow inward calcium current has been shown, the energy for which may derive from anaerobic glycolysis. Many of the observed electrophysiological changes of myocardial ischaemia can be accounted for by associated metabolic and biochemical changes. Potassium loss, decreased intracellular and extracellular pH, lactate release, catecholamine activity, "depressant" factors, and lysophospholipid release may all modulate the electrophysiological response.

## 4. METABOLIC CHANGES DURING ACUTE MYOCARDIAL ISCHAEMIA

Introduction

In the normal heart energy is derived from catabolism of substrates available to it, whether these be glucose, fatty acids, lactate, ketone bodies, pyruvate or other substances (Opie, 1968; Neely, 1974; Newsholme and Start, 1973). The preferred substrate, however, is free fatty acid in order to sustain the high energy requirements of the myocardial cell for contractile work (Crass et al, 1969; Neely, 1969). Under normal conditions an optimal balance of substrate catabolism is maintained by intricate biochemical control mechanisms.

The relative proportion of substrates actually utilised at a given time, however, is also a function of the availability of the substrates (their relative plasma concentrations), the inotropic state of the heart and hormonal influences.

Under conditions of ischaemia the metabolic response of the myocardium is determined, not only by the immediate local response to diminished perfusion, but also by the systemic metabolic response to ischaemia and altered substrate availability. A description will be given therefore of the metabolic effects of ischaemia and in particular of glucose and fatty acid metabolism in the normal heart and under conditions of ischaemia, to be followed by an account of the general systemic response to ischaemia.

## Myocardial metabolic response to ischaemia

Acute coronary occlusion results in a reduction of coronary blood flow and hence oxygen supply to the ischaemic zone. In addition reduced venous effluent flow results in accumulation of metabolites which may further modify biochemical reactions impaired by oxygen deprivation. More prolonged periods of ischaemia of from 30 minutes to one hour or more result in irreversible ultrastructural damage to the cell with swelling and destruction of mitochondria and plasma membranes and destruction of cellular organelles by release of lysosomal hydrolytic enzymes (Jennings and Ganote, 1976). These later irreversible effects will not be considered further.

Metabolic and physiological changes following experimental coronary occlusion are very rapid. The principle intracellular changes which probably take place are shown schematically in Figure 3 (after Oliver, 1975). Cyanosis and reduction of regional contraction are apparent within five seconds of occlusion (Theroux et al, 1974).

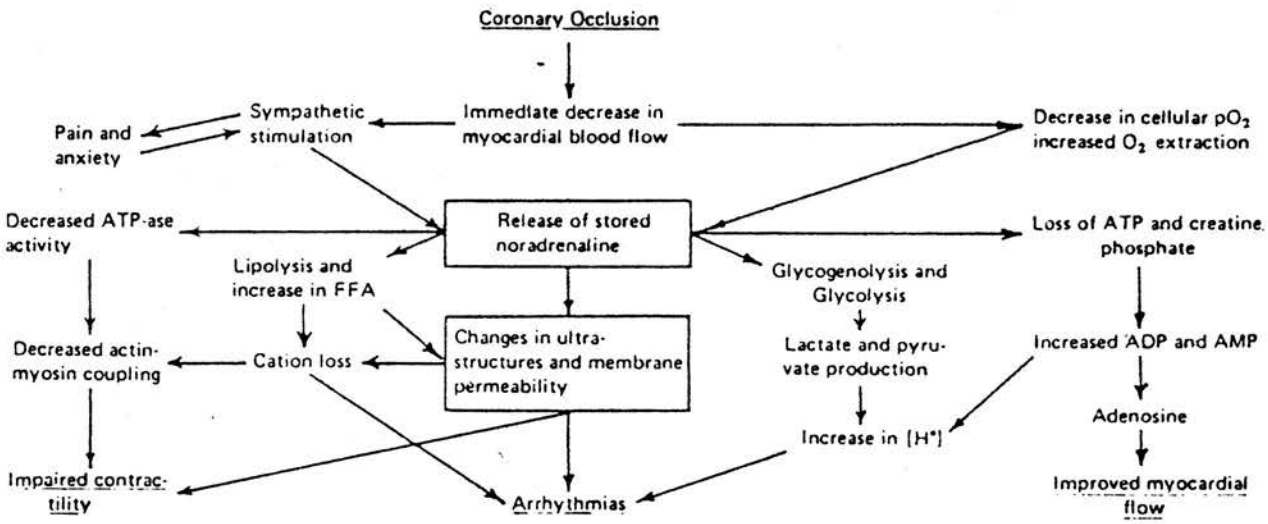


FIG. 3. An outline of the possible immediate and reversible intracellular metabolic response to myocardial ischaemia

(after Oliver, 1975)

Creatine phosphate levels fall within 30 seconds and ATP is very low within one minute of ischaemia (Braasch et al, 1968; Gudbjarnason, 1972). A rapid decline in intracellular pH accompanies these changes (Bing et al, 1973; Gudbjarnason, 1972). In addition an outpouring of potassium into the extracellular space (Burke et al, 1969; Thomas et al, 1970) and of lactate (Braasch et al, 1968), inosine and adenosine (Rubio et al, 1969) is detected, together with a fall in extracellular pH. Glycogen depletion is detectable within 30 seconds and anaerobic glycolysis stimulated (Opie, 1976). Mitochondrial swelling is observed within one minute. Catecholamines are lost from infarcting tissue with 75% disppearance within 24 hours (Russell et al, 1961) and tissue cyclic AMP levels elevated (Corr et al, 1977).

This metabolic response is dependent upon the duration of ischaemia and also the severity of flow reduction. There is evidence that a border zone (Bruyneel , 1975; Hearse et al, 1977) may exist at the edge of the ischaemic zone. A gradation may exist between well oxygenated, partially oxgenated and poorly oxygenated cells across the edge of the ischaemic area. Even within the ischaemic zone it has been suggested that heterogeneity of oxygenation of adjacent cells occurs according to collateral blood supply. Differing metabolic response might therefore be expected within partially, as opposed to poorly, oxygenated tissue. This problem will be described with respect of the metabolism of the two major substrates of the heart, glucose and free fatty acids.

Glucose metabolism

As has been described in the normally oxygenated heart, the dominant source of energy production is free fatty acid oxidation.

Glycolysis is inhibited by negative feed-back at the levels of glucose transport, hexokinase phosphofructokinase and pyruvate dehydrogenase. Although free fatty acid accumulation within the cell may directly inhibit glucose transport (Neely et al, 1969; Gross et al, 1970), the major rate limiting factor appears to be phosphofructokinase which is subject to extensive allosteric control (Neely et al, 1973). It is activated by fructose-1-6-diphosphate, ADP, AMP, $P_i$, cyclic AMP and ammonium (Neely and Morgan, 1974) and inhibited by ATP and citrate (Garland et al, 1963; Passonneau and Lowry, 1963). As fatty acid oxidation is accompanied by low levels of AMP and $P_i$ and high levels of citrate and ATP, glycolysis is inhibited. As a result of this phosphofructokinase inhibition, glucose-6-phosphate levels rise and glucose phoshorylation by hexokinase is inhibited (England and Randle, 1967). At the same time, as a result of elevated glucose-6-phosphate levels, glycogen synthesis is maintained by activation of glycogen synthetase and inhibition of glycogen phosphorylase B (Neely et al, 1974). An additional control point is provided at the level of pyruvate dehydrogenase which determines the rate of entry of pyruvate into the citric acid cycle. This enzyme is subject to allosteric inhibition by NADH and acetyl-CoA, the products of pyruvate and fatty acid metabolism (Randle et al, 1966). The rate of entry of pyruvate into the citric acid cycle is therefore linked to the rate of acetyl-CoA production from β-oxidations of fatty acids. Further control of this enzyme is achieved by a phosphorylation-dephosphorylation cycle regulated by a kinase reaction (Linn et al, 1969). Glycolysis is therefore closely linked to oxidative phosphorylation, a rise in oxidative phosphorylation and hence ATP and citrate causing a reduction in phosphofructokinase activity and hence glycolysis.

Citrate is generated, however, within the mitochondria and is totally unable to cross the inner mitochondrial membrane (Scott et al, 1972). Regulation of citric acid cycle intermediates within the cytosol is achieved by a linkage of the citric acid and cycle to the malate-aspartate cycle (Safer, 1975) which has the additional function of transferring reducing equivalents, as NADH, by a "shuttle" mechanism into the mitochondria. In turn, oxidation of NADH is essential for the maintenance of glycolysis as an oxidative reaction requiring NAD takes place at the level of glyceraldehyde-3-phosphate dehydrogenase. This will be seen to be a rate-limiting step during severe ischaemia.

During ischaemia a major shift occurs away from FFA oxidation towards glucose metabolism. Oxygen deficiency directly results in an acceleration of glycolysis by alteration of the control mechanism described. Glycolysis is stimulated within seconds of onset of ischaemia (Neely et al, 1975) due to activation of phosphorylase b kinase and rapid transformation of glycogen phosphorylase from the b to the a form, possibly as a result of a modest rise in pH due to creatine phosphate disappearance, but also due to falling ATP and glucose-6-P levels and increased levels of AMP and $P_i$ (Larner and Villar-Palasi, 1971). Several workers have shown that glucose utilisation is increased in the ischaemic dog heart by a comparison of arterial-venous differences of glucose (Brachfeld, 1967; Opie et al, 1972) or by radio-active tracer techniques (Rovetto, 1976). A distinction must be made, however, between effects in the regionally ischaemic and globally ischaemic heart. In the globally ischaemic rat heart utilisation of exogeneous glucose has been determined from $^3$H-glucose. A time-dependent

increase in glucose utilisation both from exogeneous glucose and from glycogenolysis was observed, the degree of glycolytic stimulation being related to the reduction in coronary flow (Rovetto et al, 1975). An analysis of intermediate glycolytic enzymes revealed a rate-limiting step at the level of glyceraldehyde-3-phosphate dehydrogenase. This inhibition is thought to be mediated by a fall in intracellular pH and by accumulation of NADH (resulting in turn from lactate accumulation). In hearts perfused with glucose alone, an initial stimulation of glucose uptake is followed by an inhibition (Neely, 1975). These studies were under conditions of very low flow and probably reflect an initial oxidative metabolism of glucose, followed by acceleration of anaerobic glycolysis. Continuing ATP breakdown induces proton production and hence glycolytic intermediate enzyme inhibition.

By contrast, Kubler and Spieckerman (1970) noted a 20-fold increase in glycolysis in ischaemic myocardium with the rate-limiting step at phosphofructokinase. Studies in regional, as opposed to global, ischaemia similarly suggest stimulation of glycolytic flux with relative inhibition at the level of phosphofructokinase (Opie, 1976). The mechanism of acceleration of glycolysis is poorly understood, but may result from the fall in energy levels. Decreased tissue levels of ATP and creatine phosphate and increased tissue levels of AMP and inorganic phosphate, their breakdown products, accelerate phosphofructokinase, and hence reduce tissue levels of glucose-6-phosphate, an inhibitor of hexokinase. Acceleration of hexokinase, together with a greater sensitivity of the glucose transport mechanism to insulin, can account for the observed effects.

The response to ischaemia is complicated by a number of factors. Firstly, continued oxygen availability may maintain oxidative metabolism

of glucose through the tricarboxylic acid cycle. Secondly, decreased washout of lactate, hydrogen ion and carbon dioxide may have secondary effects. Thirdly, a Pasteur effect may be operative whereby an increased uptake of glucose is converted directly into lactate output (Evans, 1933). Opie (1976) has performed a number of studies on the regional alterations in cardiac metabolism following coronary occlusion in the dog and the baboon. A very early rise (within the first minute) of tissue lactate and other glycolytic intermediates occurred. The maximum possible rate of oxidation was greatly exceeded. The accumulation of intermediates could have been accounted for by a relative inhibition of phosphofructokinase by, for example, an intracellular acidosis. Estimation of intracellular pH changes, however, showed a decrease in pH a minute later than the observed enzyme inhibition. Inhibition from another source, such as a delayed fall in ATP has therefore been suggested.

An assessment of regional metabolic changes across the ischaemic zone has been obtained by taking biopsies from four zones, viz. central infarct, peripheral infarct, border and non-ischaemic zones (Opie, 1976). In the central infarct zone collateral flow was less than 10% of normal, yet 90% of ATP was oxidatively produced meeting 20% of basal ATP requirements. In the peripheral infarct zone with flow of 20% normal, one-third of basal ATP requirements are met and in the peri-infarct boundary zone three-quarters.

The picture is therefore one of a shift towards oxidative metabolism of glucose by enhanced glycolytic flux, even in the most severely affected ischaemic zones. Final inhibition may result from excessive lactate or hydrogen ion accumulation.

Free fatty acid metabolism

In the normally oxygenated heart 60 - 70% of energy requirements are met by FFA oxidation in terms of ATP production. During exercise (or work) this proportion may rise to 90% (Neely and Morgan, 1974; Opie, 1968). Unbound FFA is taken up from plasma according to the FFA:albumin molar binding ratio and is converted into a long chain fatty acyl-CoA by means of synthetases on the outer mitochondrial membrane. This is an energy (ATP) requiring reaction and a negative feedback on it is exerted by the products of the reaction, viz. fatty-acyl CoA, AMP and pyrophosphate. The fatty-acyl CoA molecule is then transferred across the mitochondrial membrane using carnitine as a carrier. A stepwise oxidative process known as β-oxidation then takes place at the inner mitochondrial membrane such that two-carbon units of fatty acyl-CoA are converted to acetyl-CoA with reduction of INAD and 1 FAD to NADH and $FADH_2$ respectively. The nucleotides are oxidised by the respiratory chain and acetyl CoA oxidised in the tricarboxylic acid cycle. Control of this system is achieved mainly by regulation of FFA mobilisation from adipose tissue, by re-esterification of FFA to triglyceride and by negative feedback from the respiratory chain by alterations in high-energy phosphate and NADH levels (Oram et al, 1975). The inhibitory effect of fatty acid oxidation on glycolysis in the normally oxygenated heart has been discussed.

During acute myocardial ischaemia fatty acid oxidation by β-oxidation is inhibited in the rat heart (Idell-Wenger, 1976) and in the dog (Opie et al, 1973). Tissue levels of long-chain acyl CoA are increased and acetyl-CoA is decreased (Morgan et al, 1977). Although it has not yet been proven that these changes are taking place within the mitochondrion, it seems a likely assumption.

The reduced nucleotides NADH and $FADH_2$ are known to be elevated due to insufficient oxidation by the respiratory chain and would be expected to inhibit β-oxidation by a reduction of the rate of fatty acyl CoA:FAD oxidoreductase.

In addition elevated levels of fatty acyl CoA inhibit acyl-CoA synthetases (Oram et al, 1975). The uptake of FFA thus should be reduced.

The combined effect of inhibition of β-oxidation and stimulation of glycolysis has the additional effect of promoting re-esterification of FFA to triglyceride. Triglyceride accumulation has been shown in the ischaemic dog heart (Scheuer and Brachfeld, 1966) and is thought to be related to increased availability of glycerol-3-phosphate from enhanced anaerobic glycolytic flux. Increased incorporation of radio-actively labelled FFA into triglyceride can be shown under anoxic conditions (Evans, 1964).

It has been suggested that this same re-esterification process may institute a "wasteful cycle" of re-esterification and lipolysis during ischaemia (Crass, 1975). Synthesis of triglyceride requires 3ATP molecules so that a rapid turnover of the cycle could waste ATP. Attempts have been made to quantitate this cycle by determining myocardial glycerol release.

A further potentially important energy wasting system has been suggested from the studies of Shug et al (1975) who showed an inhibition of adenine nucleotide transport in ischaemic mitochondria by palmitoyl CoA. ATP produced within the mitochondria is transferred to the cystosol by an adenine nucleotide translocase system. An inhibition of this system by an accumulation of long chain fatty acyl-CoA would thus effectively trap ATP within the mitochondria.

AP synthesis is also inhibited. Results from a number of laboratories have shown reversible inhibition of adenine nucleotide translocase of liver and heart mitochondria (Shrago et al, 1976) by low concentrations of long chain acyl CoA esters. The reaction is reversed by addition of L-carnitine which removes acyl-CoA to acyl-carnitine. That this mechanism is operative in ischaemia is suggested by studies in the ischaemic dog heart (Shug et al, 1975), demonstrating a decrease in adenine nucleotide translocase activity concomitant with an increase in tissue long chain acyl CoA esters in biopsy specimens.

Elevated levels of free unbound FFA within the cytosol have not been demonstrated. If present, these and fatty acyl CoA esters have been postulated to have additional toxic effects on cell or mitochondrial membranes (Kurien and Oliver, 1970). Non-specific inhibition of high levels of FFA or fatty acyl CoA derivatives has been shown on many enzyme systems, but often in a non-specific manner (Pande and Mead, 1968). A direct toxic effect or detergent effect is possible. Alternatively, it has been suggested that such effects could be enhanced by a catecholamine induced hydrolysis of stored triglyceride (Oliver, 1975) or by complexing of unbound FFA with $Ca^{++}$ or $Mg^{++}$ necessary for the enzyme reactions of normal contraction and oxidative phosphorylation respectively. In addition FFA has been shown to uncouple oxidative phosphorylation in isolated mitochondria (Borst et al, 1962).

Systemic metabolic response to ischaemia

The external milieu of the cardiac cell may be altered by the general systemic metabolic response to ischaemia. Much of the present knowledge of these labile changes derives from clinical

studies in man following acute myocardial infarction. Important systemic changes in plasma levels of substrates, catecholamines and metabolically active hormones occur (Vetter et al, 1974; Strange et al, 1974; Oliver, 1975).

Plasma FFA and glycerol are greatly elevated and plasma glucose moderately increased for up to 48 hours following onset of acute myocardial infarction in man (Oliver et al, 1968; Vetter et al, 1974; Allison, 1972). This response is believed to relate to an increase in sympathetic activity and plasma catecholamine concentrations and accompanying elevations of cortisol, glucagon and growth hormone and reduction of insulin secretion. Plasma catecholamines are elevated within 15 minutes of coronary occlusion in man (Vetter et al, 1974) and elevated levels of catecholamines, particularly noradrenaline, have been shown in blood and urine over the following 36 hours (Valori et al, 1967; Gupta et al, 1969; Jewitt et al, 1969). The initiating event is probably reflex mediated by sympathetic and vagal afferent nerve fibres from the acutely ischaemic zone (Malliani et al, 1969). The prime stimulus may be mechanical or chemical - a change in pH or hypoxia for example.

Experimentally, a release of catecholamines, predominantly adrenaline, occurs from the adrenal medulla which may be blocked by adrenalectomy (Staszewska-Barczak and Ceremuzynski, 1968). This general increase in catecholamine activity is associated with an increased sympathetic drive to both myocardium and to the periphery.

Both noradrenaline and adrenaline and enhanced sympathetic tone stimulate adenyl cyclase in adipose tissue and enhance cyclic AMP mediated adipose tissue lipolysis (Belfrage et al, 1977). Plasma FFA levels thus are elevated by a net efflux from adipose tissue and

may be as much as three or four times normal level. FFA uptake is related, not to absolute plasma levels, but to their molar binding with albumen (Spector, 1968) in an exponential fashion. Above an FFA:albumin molar binding ratio of 3:1 the two main binding sites of albumin for FFA are saturated. The myocardium would then be expected to extract proportionately more FFA than at a lower plasma level. Such levels are readily obtained in man following myocardial infarction and experimentally by catecholamine infusion. A close relationship exists between plasma FFA and catecholamine levels (Murieson et al, 1970). Supporting this concept of enhanced lipolysis is the finding of elevated glycerol levels (Carlström, 1971) which more closely reflect the rate of tissue lipolysis. A poor correlation may exist between plasma FFA and glycerol following coronary occlusion as catecholamines have the additional effect of promoting glycogenolysis, α-glycero-phosphate production and hence re-esterification of FFA to triglyceride. Additional endocrine changes may modulate the lipolytic response to sympathetic stimulation. Cortisol enhances the reaction, as does thyroxine and growth hormone. Insulin has an inhibitory effect (Christian et al, 1969).

An early change, within 15 minutes of onset of infarction is a hypoinsulinaemia (Vector et al, 1974) leading to mild or overt hyperglycaemia as a result of either reduced glucose uptake or FFA induced reduction of insulin sensitivity (Neely, 1969). Plasma FFA levels are elevated, however, at a time when plasma glucose levels are normal and insulin levels low. The subsequent elevation of blood glucose levels is influenced by the glycogenolytic effect of adrenaline on the liver, an inhibitory effect of adrenaline on the pancreatic β cell insulin response (Porter et al, 1966) and by release of glucagon, thyroxine, cortisol and growth hormone. Elevation of

plasma cortisol has been demonstrated post-infarction (Logan and Murdoch, 1966) and is presumably a component of the general stress reaction. Growth hormone levels have been reported to be normal or elevated (Allison, 1972).

The net systemic response to ischaemia of mobilisation of FFA, glucose and plasma catecholamines, although appropriate for increased energy requirements of the normal heart, is, in many respects, inappropriate to the oxygen-deprived ischaemic cell.

## Conclusions

In the normal heart the preferred substrate for energy production is free fatty acid. Rapid metabolic changes follow experimental coronary occlusion and include reduction of tissue ATP, creatine phosphate and glycogen levels, potassium loss, release of lactate, inosine, adenosine and catecholamines. A major shift in metabolism occurs away from fatty acid oxidation towards oxidative metabolism of glucose, even in the most severely ischaemic regions by alteration of allosteric control at the level of phosphofructokinase. Re-esterification of FFA to triglyceride results in triglyceride accumulation and may institute an energy-wasting cycle of re-esterification and lipolysis. Accumulation of long chain fatty acyl CoA may trap ATP within the mitochondria by inhibition of the adenine nucleotide translocase system. In addition the metabolic response of the myocardium is modulated by the systemic metabolic response to ischaemia which, at least in man, results in elevated levels of free fatty acids, catecholamines and increased mobilisation of glucose.

## 5. SUBSTRATE MANIPULATION DURING ACUTE MYOCARDIAL ISCHAEMIA

### Introduction

It has long been known that cardiac metabolism may be influenced in the normally oxygenated heart by alterations in substrate availability. Substrate uptake is related to the concentration difference between plasma and extracellular space levels of substrate and levels within the cell. This holds above a "threshold" level of substrate concentration which has been shown to be about 1.25 mM for glucose (Opie et al, 1962) and about 350 $\mu Eq.l^{-1}$ for FFA (Carlsten et al, 1963). In the isolated heart glucose uptake increases markedly in the range 1.25 to 5 mM with a lesser rise between 5 and 10 mM and very little further rise in the 10 to 40 mM range. Similarly, over a wide range, FFA uptake is directly proportional to concentration in the perfusing medium (Scott et al, 1972). This is controlled, however, not by the absolute concentration of FFA, but by the FFA:albumin ratio as it is the "unbound" FFA which is in equilibrium with intracellular or tissue FFA (Spector, 1968). In addition uptake of unsaturated and shorter chain length fatty acids occur more readily than that of saturated or longer chain length acids (Opie, 1968).

An alteration in substrate availability can therefore influence the oxygen consumption of the heart. A complete switch from fatty acid to glucose utilisation would be expected to increase the ratio of energy produced (phosphorylation) to oxygen taken up (P/O ratio) from about 2.85 to 3.15, a change of about 10% (Opie, 1976). In practice, an increase of about 40% in oxygen consumption can be demonstrated in rat hearts given FFA as sole substrate (Challoner and Steinberg, 1966) and an increase of 15% in oxygen consumption in

the in situ dog heart by FFA elevation using triglyceride-heparin infusions (Mjos, 1971).

Under normal conditions, substrate availability to the myocardium is regulated by homeostatic mechanisms, including the proposed glucose-fatty acid cycle of Randle (1963). It is suggested that enhanced fatty acid oxidation leads to suppression of the effect of insulin on glucose transport and conversely that increased circulating glucose levels and insulin levels reduce the rate of adipose tissue lipolysis. "Preferential" substrate uptake of fatty acids, lactate, acetate or ketones has been shown over glucose uptake by the myocardium.

With this background, and with a knowledge of the alterations of substrate levels during acute ischaemia (see Section 4), ideas regarding possible beneficial effects of substrate manipulation during acute myocardial ischaemia have emerged. Historically, two major hypotheses have emerged - the glucose hypothesis and the fatty-acid hypothesis.

## The glucose hypothesis

The importance of glucose to the metabolism of the heart has been known since the turn of the century. Locke (1907) observed that dextrose markedly influenced the beat of the isolated perfused frog or rabbit heart. It was soon advocated for clinical cardiological use (Goulston, 1912; Büdingen, 1914) as a myocardial fuel. Following the discovery of insulin, therapeutic regimes of glucose and insulin were suggested for the management of angina pectoris (Smith, 1933).

Only in the last 15 or 16 years has experimental evidence appeared that glucose, or glucose and insulin, or glucose-insulin-potassium mixtures (GIK) may have some beneficial effect on the survival of ischaemic myocardium or incidence of cardiac arrhythmias. The topic remains controversial to this date.

Sodi-Pallares (1962, 1963) suggested that hypertonic glucose might exert a "polarising" effect on the ischaemic cell by enhancing potassium uptake into cells partially depolarised by potassium leakage and that this effect should be beneficial in the treatment of arrhythmias. It was thought that potassium loss and hence "depolarisation" of cells in the ischaemic area was the dominant factor in the genesis of arrhythmias (Sewell et al, 1955; Harris, 1966). His hypothesis was based upon the finding of significant reductions in arrhythmias, conduction block, Q wave evolution and ST-segment elevation following 2-stage coronary ligation in the dog by "polarising therapy" of glucose, insulin and potassium. Laborit and Huguenard (1963) confirmed that treatment of rabbits with glucose and insulin prevented potassium chloride induced ventricular fibrillation. Potassium release from areas of infarction correlated with arrhythmias (Harris et al, 1960) and glucose-insulin infusion reduced both experimental arrhythmias and potassium release in the coronary sinus (Cherbakoff et al, 1957). Similarly, in man "polarising therapy" over a period of three to seven days, post-infarction reduced the frequency and duration of arrhythmias, the incidence of residual angina or aneurysm and hastened the disappearance of ST-segment ischaemic injury on the electrocardiogram (Sodi-Pallares et al, 1962).

Attempts to reproduce these findings were not uniformly successful. Mittra (1965, 1967) performed two clinical trials and

concluded that polarising therapy significantly reduced arrhythmic mortality within 14 days of infarction. Using Mittra's GIK regime, however, Pilcher et al (1967) could demonstrate no clinical benefit. As many studies failed to confirm a beneficial effect (Fletcher et al, 1966; Malach 1967; Pentecost et al, 1968) as found it (Ponce de Leon et al, 1962; Sodi-Pallares et al, 1969; Calva et al, 1965). In an attempt to resolve this problem a multicentre trial was set up by the Medical Research Council (1968). No significant difference was found in mortality at 28 days after infarction between control and glucose-insulin-potassium groups. Considerable variations occurred in different centres, however, and glucose was given orally and in smaller doses than advocated by Sodi-Pallares and treatment commenced as late as 48 hours after the initial event. Diet was unrestricted and propranolol given for arrhythmias. Arrhythmia analysis was not performed.

Despite major criticisms of each of the clinical trials, an air of doubt as to the clinical effectiveness of glucose-insulin therapy was created. Independent experimental evidence confirmed that glucose-insulin-potassium could reverse the cumulative potassium loss from ischaemic myocardium and reduce the incidence of ventricular tachycardia and fibrillation, at least within the first four hours after coronary occlusion in the dog (Regan et al, 1967). A lack of correlation, however, was shown between potassium loss in the coronary venous effluent and the incidence of post-occlusion arrhythmias (Thomas et al, 1970). In addition a measurable loss of tissue potassium was not demonstrated until one or two hours after the onset of ischaemia, well beyond the onset of the first phase of arrhythmias (Jennings et al, 1957).

Interest was renewed in the potential usefulness of glucose therapy by a consideration of possible beneficial metabolic effects in ischaemic myocardium. A metabolically orientated "glucose hypothesis" was therefore put forward by Opie (1970). It was suggested that as, under conditions of anoxia, the metabolism of the heart is diverted from FFA metabolism to anaerobic glycolysis, and that under these conditions survival may be dependent upon the rate of utilisation of glucose or glycogen, then under conditions of limited oxygen availability, any measure to promote glucose utilisation should enhance the survival of the heart. Such measures might then include an increase in extracellular pH, a high circulating glucose concentration, addition of insulin or the removal of inhibitors of glucose metabolism such as free fatty acids or ketone bodies. Opie makes clear, however, that caution must be exercised in extrapolating directly from the anoxic perfused heart to the regionally ischaemic heart. Calculations show that anaerobic glycolysis of glucose to lactate could only provide about one-hundredth of the ATP requirements of the normal beating heart. A stimulation of both aerobic and anaerobic glycolysis during ischaemia must therefore be postulated. Evidence that this is indeed the case has appeared over the last five years.

Alternative modes of action of glucose during ischaemia are possible (Opie, 1970). An improvement in cardiac transmembrane potential changes (vide infra) could be anti-arrhythmic. Beneficial metabolic effects could arise as a result of insulin alone, or indirectly by a suppression of circulating plasma FFA levels. An increase in plasma osmolality by hyperglycaemia could have a mannitol-like effect in reducing ischaemic injury. Finally, an

elevation in plasma volume might stimulate an inotropic left ventricular response.

Metabolic effects of GIK of reducing plasma FFA levels (Moffit et al, 1973) and arterial coronary sinus FFA levels (Stanley et al, 1974) have been confirmed during acute ischaemia in man.

Effect of glucose on the extent of myocardial ischaemic injury

The degree of myocardial ischaemic injury following experimental coronary artery occlusion may be diminished by glucose or glucose-insulin-potassium infusion. Maroko et al (1972) commenced therapy 30 minutes after coronary occlusion in the dog and compared the degree of ST-segment elevation 15 minutes after occlusion in control, glucose and glucose-insulin-potassium treated groups of animals with myocardial creatine phsophokinase (CPK) activity in the myocardium 24 hours later. Less CPK depletion was observed after glucose-insulin-potassium or glucose therapy than in the control group. The effect of glucose alone was slightly less than that of glucose-insulin-potassium. Sites which exhibited ST-segment elevation 15 minutes after occlusion were found on analysis at 24 hours to be histologically normal in only 3% of specimens in the control group, compared to 30% in the glucose-treated group and 37% in the glucose-insulin-potassium group. These findings were confirmed on electron microscopy.

These findings have been confirmed and extended by analysis of metabolic changes in tissue biopsy samples from the baboon heart within the first hour of coronary occlusion (Opie et al, 1975). Glucose-insulin-potassium infusion halved CPK and glycogen depletion

and increased tissue contents of high energy phosphate in the ischaemic zone and increased glycogen and lactate concentrations in the peri-infarct zone. The tissue sodium/potassium ratio was also improved. Similar effects were obtained whether high or low dose glucose infusions were given. Similar findings were obtained in the dog (Opie et al, 1976). Glucose-insulin-potassium was infused over a six hour period commencing 30 minutes after acute coronary artery occlusion. Arterial levels of just over 10 mM glucose were attained. A fall of ST-segment elevation in the central infarct zone was acclerated, whereas a rise in the peri-infarct zone was prevented. Tissue contents of glycogen, ATP and lactate were increased and potassium/sodium ratio improved, particularly in the border peri-infarct zone. In addition arterial-venous differences of glucose were increased and of FFA:albumin ratio decreased. These effects were thought to be mediated by an enhancement of aerobic glycolysis, particularly in the border or peri-infarct zone.

Studies in isolated or globally ischaemic heart models have not confirmed these findings. Rovetto (1975) found neither protection nor increased glycolysis in ischaemic rat hearts treated with glucose and insulin. In globally ischaemic perfused working swine hearts Liedtke (1976) found significant decreases in myocardial oxygen consumption, fatty acid oxidation and high-energy phosphate stores during ischaemia in control and glucose-insulin treated hearts. Treated hearts showed greater reductions in mechanical performance and ten minutes less average survival time. Important differences may exist, however, in the metabolism of globally and regionally ischaemic hearts. Washout of accumulated

lactate or potassium, for example, may be delayed in global ischaemia and lead to a secondary inhibition of glycolysis. In the ischaemic rat heart, however, glucose diminishes fatty acid induced increases in enzyme release and changes in mitochondrial function and morphology (de Leiris et al, 1975; Lochner et al, 1976; Waldenstrom and Hjalmarson, 1977).

Norris et al (1978) were unable to confirm the findings of Maroko and Opie in the dog. No beneficial effect of glucose-insulin-potassium was found on ST-segment elevation and CPK depletion when treatment was commenced 15 minutes after occlusion, although transient significant increase in arterial-local venous differences of glucose occurred after 15 minutes of treatment and lactate output was doubled. A higher coronary occlusion and greater elevation of blood glucose levels were used, however, and beneficial effects may have been offset by the increased severity of ischaemia or a blood volume expansion effect.

## Electrophysiological effects of glucose

Beneficial electrophysiological effects of glucose or glucose-insulin-potassium infusion, in terms of reduction of incidence in arrhythmias and diminution of ST-segment changes during ischaemia, have been described above.

Early animal studies were directed towards confirming the so-called "polarising" effect of glucose during ischaemia. Regan et al (1967) demonstrated that glucose reduced both potassium egress from the great cardiac vein and the incidence of ventricular tachycardia following coronary occlusion in the dog during a one hour infusion. A later study (Burke et al, 1969), using a four hour infusion of glucose, again showed diminished potassium loss,

enhanced glucose uptake and reduced incidence of ventricular fibrillation after electronic thrombus production in the left anterior descending coronary artery. At variance with the "polarisation" theory, however, was the finding that reversal of potassium egress during ischaemia could be similarly achieved by an infusion of hypertonic saline without an increase in glucose uptake. The possibility that neural stimulation and noradrenaline release at this time might promote potassium uptake was postulated.

Evidence for a direct electrophysiological effect of glucose on the transmembrane action potential arose from McLeod and Daniel (1965) following the chance observation that the electrical activity of cat papillary muscle became increasingly sensitive with time to relative oxygen lack in a medium containing 5 mM glucose, but not in one containing 50 mM. They subsequently showed that the shortening in action potential duration following anoxia was abolished by substituting 50 mM for 5 mM glucose. A small hyperpolarising effect also occurred with increase in action potential amplitude.

The effect was attributed initially to an effect on potassium transport and hence potassium currents of repolarisation as it was possible to partially duplicate it by xylose or 2-deoxyglucose. Further studies (McLeod and Prasad, 1969) showed that the degree of action potential shortening was directly related to the glucose concentration in the medium. Furthermore, the changes induced by glucose during anoxia were blocked by phlorizin and this block could be reversed by addition of insulin. The effect of glucose could not be completely reproduced by xylose, 2-deoxyglucose, arabinose, D-galactose and other non-metabolised sugars. It was

suggested therefore that this effect of glucose might be mediated by a stimulation of anaerobic glycolysis. Changes in tissue ATP and action potential shortening were found to be in parallel with lactate production during anoxia (McDonald and McLeod, 1971).

Pyruvate partially reversed the shortening of action potential duration under normoxia or anoxia induced by iodoacetate. Similarly, glucose, but not pyruvate, was effective in reversing action potential shortening induced by uncoupling of oxidative phosphorylation with dinitrophenol or sodium cyanide (Prasad and McLeod, 1969). These data support the suggestion that anaerobic metabolism of either glycogen or exogenous glucose is capable of maintaining normal transmembrane electrical activity in the papillary muscle. It was suggested further that the changes could be mediated by a metabolic dependence of the selective ionic permeabilities of the slow inward current, rather than an increase in potassium conductance. It was shown, for example, that $^{42}K$ efflux was not increased when the action potential was rapidly shortening during anoxia, and that manganese, which blocks the slow inward calcium current, reduced action potential duration of anoxic guinea-pig ventricular muscle in 50 mM glucose medium (McDonald and McLeod, 1972). The absence of a large loss of resting potential during anoxia was surprising and could be accounted for by a powerful stimulation of the electronic sodium pump (ouabain sensitive). Surprisingly, addition of insulin had little or no effect on the abbreviated action potential during anoxia (MacLeod and Prasad, 1969). Insulin-induced glucose uptake may have been diverted, however, to glycogen synthesis (Villar-Palasi and Larner, 1961).

Alternative explanations for these possible slow current effects have been put forward. Hyde (1972) from work in isolated

cultured myocardial cells suggests that ATP could chelate subsarcolemmal calcium ions, so reducing free available calcium and increasing the driving force for the slow calcium flux. Alternatively, a metabolically dependent control of calcium channels regulated by cyclic AMP derived from ATP is possible (Schneider and Sperelakis, 1974).

Further support for metabolic dependency of the slow inward calcium flux is given by studies in potassium depolarised cat papillary muscle exposed to adrenaline (Wojtczak and Russell - unpublished). Slow potentials induced by field stimulation decreased in amplitude and duration during anoxia, the effect being partially reversed by increasing glucose from 5 to 35 mM.

Studies on anoxic human papillary muscles isolated at cardiac surgery have confirmed a reversal of action potential shortening, increased overshoot and resting membrane potential after addition of glucose (Prasad and Callaghan, 1969). The effect was increased or decreased by low or high doses of ouabain respectively (Prasad, 1968), probably related to a stimulation or depression of membrane ATP'ase and hence replenishment of intracellular potassium levels.

An extrapolation, however, from such in vitro effects to myocardial ischaemia and arrhythmogenesis must be highly speculative.

A lack of knowledge concerning electrophysiological effects of glucose during acute ischaemia prompted some of the experimental studies described in this thesis.

## The fatty acid hypothesis

Kurien and Oliver (1970) proposed the hypothesis that elevated levels of plasma FFA may be harmful or indeed toxic to the ischaemic myocardium, and that this may be a factor in the

initiation of ventricular arrhythmias. It was suggested that intracellular FFA levels may rise as a result of both elevated plasma FFA levels and inadequate glucose uptake owing to insulin suppression and that an accumulation of free "unbound" FFA within the cell could lead to detergent effects on the cell membrane with cation loss and development of ectopic pacemaker activity. It was not considered that FFA per se necessarily would induce arrhythmias, but that the combination of elevated FFA, together with some additional stimulus such as increased catecholamine activity or potassium leakage from the cell might do so.

The corollary of this hypothesis is that if some arrhythmias are FFA induced, then therapeutic benefit may be obtained by suppressing tissue mobilisation of FFA and by diverting ischaemic myocardial metabolism towards energy production from glucose.

The background to this hypothesis was the clinical finding that plasma FFA may be considerably elevated in the first 24 hours of acute myocardial infarction (Kurien and Oliver, 1966). Furthermore, those patients with very high plasma FFA levels suffered more arrhythmic deaths. A significant correlation was found between high levels of plasma FFA and arrhythmias and deaths in a series of 200 consecutive patients with acute myocardial infarction. Thirty-three per cent of patients with plasma FFA greater than 1200 $\mu Eq.l^{-1}$ died, compared with 5% of patients with plasma FFA levels below 80 $\mu Eq.l^{-1}$ (Oliver et al, 1968).

A cause and effect relationship between elevated FFA alone and arrhythmias developing during ischaemia in man has not yet been established and increased catecholamine activity or decreased insulin availability are both likely to be important

contributory influences. Experimentally, however, elevation of plasma FFA by infusion of triglyceride-heparin following acute circumflex coronary artery occlusion was followed after 20 minutes by bursts of ectopic activity, including ventricular tachycardia (Kurien and Oliver, 1970). The effect was reversed by administration of protamine sulphate which abruptly lowered plasma FFA levels.

Further circumstantial evidence has been quoted in favour of the hypothesis that the known increase in myocardial oxygen consumption induced by FAA in the isolated rat heart; the association between a high metabolic rate and elevated FFA in conditions such as thyrotoxicosis and diabetic ketosis; and the known detergent effects of unbound FFA on cell membranes.

An association between elevated plasma FFA and arrhythmias has been confirmed in several clinical studies (Gupta et al, 1969; Reiman and Schwandt, 1971; Prakash et al, 1972), although the response could merely reflect a general metabolic response to the stress of infarction. Other studies, by contrast, have failed to show any such association (Nelson, 1970; Ravens, 1972). Rutenberg et al (1969) showed no correlation in 78 patients between initial fasting FFA and development of arrhythmias, cardiogenic shock or late death, although there was a tendency for FFA to be elevated at the time of any complication such as heart block, junctional rhythm or ventricular premature beats. In addition no increased incidence of arrhythmias was demonstrated by Nelson (1970) in 24 patients treated with heparin (which has the effect of elevating plasma FFA) within the first 24 hours of myocardial infarction, although a definite correlation was shown with plasma noradrenaline.

Early experimental work was equally controversial. Opie et al (1971) were unable to confirm the experimental findings of Kurien and Oliver (1970) in the open chest dog preparation. A controversy was stimulated by the apparently contradictory findings of these two experimental studies. Important differences were present, however, in the experimental models utilised. Opie et al used open chest occlusion of the left anterior descending coronary artery in the greyhound, whereas Kurien performed a closed-chest balloon occlusion of the circumflex coronary artery in the sheepdog. The plasma albumin concentrations of the Edinburgh sheepdog (c. 2 g/100 ml) were low, compared with those of the greyhound (c. 4 g/100 ml). In view of the relationship between FFA uptake and FFA:albumin molar binding ratio (Spector, 1968), it is possible that for given levels of plasma FFA actual myocardial FFA uptake in the sheepdog could have been twice that of the greyhound. In addition the local sympathetic nerve supply to the ischaemic zone would be damaged by direct coronary artery ligation, whereas a balloon occlusion would leave this intact. More difficult to explain is the delay in onset of arrhythmias in Kurien's group after elevation of plasma FFA. A delayed toxic effect of FFA or of activated fatty acyl CoA must be postulated.

Largely as a result of the fatty acid hypothesis, further experimental and clinical work has been undertaken to assess the effects of manipulation of substrate levels of FFA or myocardial function, the extent of ischaemic injury, arrhythmogenesis and electrophysiological properties during acute myocardial ischaemia.

Effect of FFA on the extent of myocardial ischaemic injury

Serious difficulties have arisen experimentally in the direct determination of the effect of raised plasma FFA during ischaemia. FFA cannot be infused directly on account of induction of massive haemolysis and hypotension (Riemersma, 1974) or of thrombosis (Connor et al, 1963). Intravenous infusion of the sodium salts of FFA may cause conduction defects (Soloff, 1970). Infusion of albumin bound FFA cannot increase plasma levels of FFA sufficiently high for experimental purposes on account of the rapid turnover of FFA in the body. In addition studies using triglyceride-heparin infusion must be viewed with caution in the light of recent studies from our laboratory showing gross over-estimation of true plasma FFA levels due to excessive in vitro plasma lipolysis (Riemersma et al, 1978). The more direct approach therefore of inhibition of catechol-stimulated lipolysis has been widely used to study the effects of reduction of elevated plasma FFA levels.

An increase in arterial FFA in the normal dog increases myocardial oxygen consumption without influencing the mechanical activity of the heart (Mjøs, 1971). By inhibiting catecholamine-induced lipolysis with β-pyridyl carbinol as much as a 30% reduction in myocardial oxygen consumption attributable to a fall in plasma FFA may be obtained (Mjøs, 1971). This is independent of any chronotropic or inotropic effect. Similar studies performed during acute myocardial ischaemia (Kjekshus and Mjøs, 1973) demonstrate a significant reduction in ST-segment elevation when β-pyridyl carbinol is infused in combination with isoprenaline, compared with infusion of isoprenaline alone. This was not associated with any haemodynamic disturbance and correlated with subsequent measurements

of myocardial CPK activity. Triglyceride-heparin infusion increased the number of recording sites showing ST-segment elevation, although the summed ST-segment elevations were not increased. Reduction of FFA availability to the ischaemic myocardium was considered partially protective therefore. Similar reduction in ischaemic induced ST-segment elevation in the dog has been shown with the antilipolytic agents nicotinic acid, sodium salicylic acid (Vik-Mo and Mjøs, 1976) and p-chloro-phenoxy-isobutyrate (Mjøs et al, 1976). Infusion of lipid-free albumin induces transient reduction of ST-segment elevation within the first few minutes of acute myocardial ischaemia (Miller et al, 1976), the effect being attributed to a reduction of available "unbound" FFA by binding to available sites on the infused albumin molecules. The experimental effects of triglyceride-heparin infusion are less clear cut. Kjekshus and Mjøs (1972) showed that although triglyceride-heparin infusion did not influence the fall in myocardial oxygen consumption induced by acute myocardial ischaemia, there was a greater depression of regional contractility in the ischaemic zone at a given myocardial oxygen consumption after infusion of triglyceride-heparin. This finding is consistent with the observation of decreased contractility of the hypoxic rat papillary muscle following in vitro superfusion with fatty acids in high concentration (Henderson et al, 1970).

Clinical studies have shown significant beneficial effects of antilipolytic agents during the first eight hours of onset of acute myocardial infarction. A reduction in ST-segment elevation is achieved within the first six hours of acute infarction following the lowering of plasma FFA levels by the nicotinic acid analogue

5-fluoro-nicotinic acid (Russell and Oliver, 1978). Similar effects in man on ST-segment elevation, together with a reduction in spatial QRS infarct vector evolution follow treatment with β-pyridyl-carbinol (Simonsen and Kjekshus, 1978), strongly suggesting a protective effect on infarcting myocardium of antilipolytic therapy.

This antilipolytic effect may be greater in the presence, rather than the absence, of catecholamines. Relatively small increases in myocardial oxygen consumption follow elevation of plasma FFA alone (Simonsen and Kjekshus, 1978; Most et al, 1973), whereas in the catecholamine stimulated heart 45% of the increase in myocardial oxygen consumption can be accounted for by elevation of FFA (Kjekshus, 1978), compared with 29% from an increase in contractility and 23% from an increase in heart rate. In addition β-pyridyl carbinol is not effective in reducing epicardial ST-segment elevation following acute coronary artery occlusion in reserpinised animals (Kjekshus, 1978).

It has been suggested that antilipolytic therapy may be effective in breaking a vicious circle (Opie et al, 1977), whereby increasing severity of ischaemia may lead to further complications and enhanced sympathetic drive, so increasing plasma FFA levels and hence reducing glucose inhibition in the heart and further increasing the severity of ischaemia. Both catecholamine release and plasma FFA elevation would increase tissue damage.

Electrophysiological effects of FFA

Evidence concerning electrophysiological effects of FFA is in general conflicting.

Elevation of FFA by glucagon in the goose induces cardiac

arrhythmias and myocardial necrosis (Hoak et al, 1969). In the normally oxygenated perfused rat heart ventricular arrhythmias can be induced if a sufficiently high FFA:albumin molar binding ratio is attained (Willebrands et al, 1973). High and low albumin solutions complexed to oleate or palmitate gives rise to arrhythmias at molar binding ratios of 3:1 or 5:1. Conversely, elevation of serum FFA by infusion of triglyceride-heparin had no effect on ventricular fibrillation threshold determinations either before, immediately after or three days after acute coronary ligation in the dog (Kostis et al, 1973). A reduction of fibrillation threshold has been found, however, between 120 and 180 minutes after coronary ligation following triglyceride-heparin (Takano, 1976).

A suggestion that FFA might exert some direct membrane effect came from the work of Wasilewska-Dziubinska et al (1975) who observed marked changes in the shape of epicardial action potentials in the isolated Langendorff-perfused guinea-pig heart following addition of 0.5 mM palmitate complexed to albumin 4 g per cent. Shortening of phases 2 and 3 of the action potential were observed during combined infusion of glucose and palmitate which led in many instances to ventricular fibrillation. Their control albumin preparation, however, contained significant amounts of FFA and effects observed after addition of palmitate were no different from those obtained after removal of exogenous substrate. The authors subsequently have withdrawn conclusions made in this paper (personal communication). Ravens and Ravens (1976) could show no effect of linoleate on the action potential duration of isolated superfused guinea-pig papillary muscle under

conditions of normoxia or hypoxia. Similarly, no significant effect of oleic acid in a molar binding ratio of 3:1 can be shown on the action potential duration of superfused dog papillary muscle or ventricular trabeculae under conditions of normoxia or hypoxia (author - unpublished). The effect of palmitate (0.6 mM) complexed to albumin (1 per cent) on epicardial ventricular action potentials in the presence of glucose was examined in the Langendorff perfused guinea-pig heart by Cowan and Vaughan Williams (1977). No effect on action potential duration was found in normoxia or in severe hypoxia, but an exacerbation of shortening in action potential duration was observed in moderate hypoxia (25% $O_2$) and during successive periods of reduced flow perfusion. In addition the decline in action potential duration occurring in the absence of glucose was partially reversed by palmitate. By contrast, a concentration dependent decrease of action potential duration has been shown with both linoleate and palmitate complexed to albumin in superfused guinea-pig papillary muscle under conditions of normoxia (Lüderitz et al, 1976). Addition of free fatty acids had an additional effect on action potential shortening during hypoxia and addition of glucose partially reversed a fatty acid induced reduction of refractory period.

It is possible that certain of these discrepancies may be accounted for on the basis of difficulty in FFA-albumin binding. Unbound FFA in high concentrations might then be expected to exert a powerful non-specific detergent effect on the cell membrane. Electrophysiological effects have been demonstrated, however, by the water soluble fatty acid octanoic acid. Spontaneous firing of isolated ventricular muscle strips is inhibited, whereas

action potential duration and upstroke velocity of Purkinje fibres are reduced (Borbola et al, 1974). It is suggested that these changes could be arrhythmogenic. Both free and albumin bound arachidonic acid, the fatty acid precursor or prostaglandins $E_2$ and $F_2$ have a dose-dependent positive chronotropic and modest inotropic effect on isolated guinea-pig atria not shown by linoleic or oleic acid (Borbola et al, 1977). The relevance of such in vitro studies to changes occurring during ischaemia or the initiation of arrhythmias must remain speculative.

Experimental animal studies and more recent clinical work suggest that at least inhibition of lipolysis by antilipolytic therapy has some antiarrhythmic effect. Kurien et al (1971) reported that 5-fluronicotinyl alcohol suppressed catechol induced ventricular arrhythmias provoked by acutely elevating FFA in dogs subjected to acute coronary occlusion during the first three hours of ischaemia. Using this same antilipolytic agent during the later delayed phase (Harris stage III) of arrhythmias in anaesthetised dogs, Smith and Duce (1974) showed a significant correlation between reduction in incidence of ventricular arrhythmias and fall in plasma FFA levels. Variations in mean FFA levels accounted for between 33 and 50% of the variation in mean abnormal beats.

In man 5-fluoronicotinic acid is effective in reducing the incidence of ventricular tachycardia within the first six hours of onset of symptoms of acute myocardial infarction, but only in that subgroup of patients with the greatest degree of reduction of plasma FFA (Rowe et al, 1975). This effect was not associated with any haemodynamic effect or change in plasma glucose or total catecholamine

levels. The possibility of some direct membrane effect of the drug is unlikely following the finding of an absence of electrophysiological effects of the parent compound nicotinic acid on the cardiac action potential (Beresewicz and Wojtczak, 1976).

The composition, rather than the absolute level of FFA, may be of importance. Ravens and Ravens (1972) found an elevation of plasma FFA in patients following myocardial infarction with ventricular arrhythmias, but relatively lower levels of oleic acid and higher levels of linoleic acid occurred in the arryhthmic compared with the non-arrhythmic groups. Differential electrophysiological effects of different chain length fatty acids is thus a possibility. Supporting this concept is the observation that dietary alteration of cardiac phospholipid fatty acid content can reduce isoprenaline tolerance in the rat (Gudbjarnason and Oskarsdottir, 1977). Mortality from isoprenaline stress was doubled by cod liver oil feeding in association with increased tissue levels of docosahexanoic acid and decreased levels of oleic and linoleic acids. It has been suggested further that docosahexanoic acid could participate in the formation of cation-conducting transmembrane channels, in particular of the sodium channel. The requisite physical properties for such a channel are possessed by two opposite fatty acid molecules in spiral or helical form (Gudbjarnason et al, 1978). The electrophysiological behaviour of the ischaemic heart thus may be influenced by alterations of membrane stucture.

## Conclusions

Myocardial substrate uptake is a function of plasma substrate concentrations. Myocardial metabolism may be influenced therefore by altered substrate availability. In general terms, it has

been suggested that glucose is "good" and free fatty acids are "bad" in terms of their metabolic effects during myocardial ischaemia.

Clinical studies with glucose or glucose-insulin-potassium remain controversial. Experimental studies, similarly, have shown both protective effects on arrhythmias and infarct size and a lack of effect. In vitro electrophysiological studies, however, show a normalisation of anoxic changes in the cardiac action potential by glucose.

An association exists between elevations of plasma FFA and arrhythmogenesis, but a cause and effect relationship is not established. Antilipolytic therapy can reduce myocardial oxygen consumption and ischaemic injury and is clinically anti-arrhythmic. Evidence regarding direct electrophysiological effects of FFA is conflicting.

# METHODS AND RESULTS

# METHODS AND RESULTS

## 6. INTRODUCTION

Evidence has been presented that electrophysiological abnormalities during acute myocardial ischaemia can be related to mechanisms of arrhythmogenesis (Section 2). Normal cardiac electrical activity, however, is metabolically dependent and many of the electrophysiological changes observed during acute myocardial ischaemia may be related to metabolic abnormalities (Section 3). Further, the metabolic response to acute myocardial ischaemia (Section 4) may be modulated by alterations in substrate availability by manipulation of plasma substrate levels (Section 5). Beneficial effects upon both myocardial ischaemic injury and arrhythmogenesis have been described following infusion of glucose (or glucose-insulin-potassium mixtures) or following reduction of plasma free fatty acid levels by antilipolytic therapy. Many reported effects have not been confirmed, however, by independent investigations (Section 5).

This thesis addresses itself therefore towards an investigation of the possible electrophysiological effects of potentially beneficial substrate manipulations during acute myocardial ischaemia which may relate to the genesis of lethal arrhythmias. Of particular interest is the potential application of metabolic intervention in the management or prophylaxis of the lethal arrhythmias resulting in sudden death from coronary heart disease in man.

The major hypothesis of this thesis is that substrate manipulation (by elevation of plasma glucose or reduction of plasma free fatty acid levels) may ameliorate the myocardial metabolic response to ischaemia and hence diminish electrophysiological abnormalities of importance in the genesis of lethal arrythmias.

A corollary of this hypothesis is that an understanding of the natural history of electrophysiological abnormalities during acute myocardial ischaemia and of their relation both to mechanisms of arrhythmogenesis and abnormalities of myocardial metabolism are central to an understanding of the mode of action of metabolic intervention.

Studies in man are impractical in view of the virtual impossibility of obtaining patients and the necessity for invasive measurement. An experimental model or series of models have therefore been developed and utilised based upon the following considerations:-

a) simulation or partial simulation of the mechanisms of pathogenesis of the lethal arrhythmias responsible for sudden death early after onset of symptoms of infarction in man.

b) ease of manipulation of substrate availability.

c) reproducibility of data during acute myocardial ischaemia to allow a comparison of intervention with control.

d) preference for analysis in the in situ beating heart subject to neurogenic and systemic metabolic influences.

e) analysis of electrophysiological variables likely to be important determinants of arrhythmogenesis.

f) analysis of the inter-relationships between any effects of substrate manipulation upon electrophysiological abnormalities and associated alterations in ischaemic myocardial metabolism or blood flow or in secondary systemic haemodynamic or metabolic effects.

The choice of the open-chest anaesthetised dog preparation was determined by a number of factors. Firstly, an early phase of enhanced vulnerability to arrhythmogenesis has been demonstrated following acute coronary occlusion in the dog (Harris, 1952) which is maximal after five minutes or so of ischaemia and which is believed

to relate to the early phase of arrhythmogenesis following onset of symptoms of acute infarction in man (Bigger et al, 1977). Secondly, substrate manipulation is easily achieved by intravenous infusion. Thirdly, several workers have demonstrated the effects of a number of interventions on myocardial ischaemic injury using the model of repetitive sequential coronary occlusions in the dog (Maroko and Braunwald, 1972; Kjekshus and Mjøs, 1972; Mjøs et al, 1976a, 1976b). Apparently reproducible data is assumed over the period of successive short periods (10 or 15 minutes) of myocardial ischaemia. A comparison of effects during control coronary occlusions with effects during an interposed coronary occlusion in the presence of some intervention is thus possible. Fourth, an analysis of potential anti-arrhythmic effectiveness of an intervention during acute myocardial ischaemia is only tru ly meaningful in the in situ beating heart. Although much useful information has been gained from electrophysiological studies in the superfused isolated tissue preparation, much of the application of this knowledge to the intact organism is by inference only. Similarly, the use of an isolated perfused heart preparation precludes an analysis of potentially important effects resulting from neurogenic or systemic endocrine, metabolic and haemodynamic effects or secondary alterations in coronary flow and perfusion. Many such studies involve induction of global ischaemia, albeit at graded levels of coronary flow. The electrophysiological and metabolic effects of regional ischaemia, as follows occlusion or partial occlusion of one part of the coronary vascular tree, are known, however, to differ markedly from those of global ischaemia. Inhomogeneities of metabolite release or sensitivity to neural stimulation or catecholamines may be considerably greater in regional than global ischaemia. These

factors are taken into account in a preparation involving coronary occlusion in the in situ beating heart. Fifth, the dog heart has been considered to show similar metabolic and electrophysiological responses to ischaemia to those reported in the primate (baboon) heart (Opie et al, 1975). Difficulties are encountered with the sheep heart due to its peculiar coronary vasculature and in the pig heart due to both a lack of intercoronary anastamoses and the peculiar anatomy of the specialised conducting system, which, unlike in man and the dog, penetrates to the epicardial layers of myocardium. In smaller mammals, such as the cat, regional ischaemia is difficult to induce, metabolic determinations hampered by size and electrophysiological findings difficult to relate to the higher mammal on account of the peculiar nature of its cardiac action potentials. Nevertheless, considerable anatomical variability in coronary arterial collateral blood vessel formation in the dog renders the production of a reproducible degree of myocardial ischaemic injury following coronary occlusion almost impossible. Studies must therefore be designed to allow for each animal to act as its own control following coronary occlusion. Finally, the size of the dog heart has advantages in permitting cannulation of the local vein draining from the ischaemic region for blood sampling purposes and estimation of metabolic gradients of substrates. In addition experience has been gained with the use of radioactively labelled microspheres in the determination of regional myocardial blood flow in the dog. Such procedures, although technically possible, are considerably more difficult in smaller animals, such as, for example, the cat.

The choice of dog is of importance. It has been mentioned that certain discrepancies between experimental findings of the

effects of elevated plasma free fatty acids on arrhythmogenesis during acute myocardial ischaemia may be accounted for by the choice of the sheepdog in one series of studies (Kurien and Oliver, 1970) and the greyhound in another (Opie et al, 1971). Plasma albumin concentrations of the former were half those of the latter group, so that at similar plasma free fatty acid concentrations the molar binding ratio of fatty acid was halved in the greyhound. In view of ease of availability the Scottish mongrel sheepdog was chosen for these studies.

Induction of regional ischaemia by experimental coronary occlusion is believed to simulate the acute initiating event of impending arrhythmogenesis or infarction in man (Bigger et al, 1977). The mode of induction of occlusion, however, deserves consideration. The use of a coronary clip occlusion of the left anterior descending coronary artery in the open-chest preparation has been chosen for the studies presented in this thesis. The use of an open-chest preparation is largely determined by the need for direct application of multiple electrodes for electrophysiological recording and for direct blood sampling and microsphere injection. Thoracotomy, however, produces considerable haemodynamic effects and exposure of the heart with pericardectomy may have reflex effects and result in local cooling. Again, dissection of the coronary artery to allow for application of an occlusive clip is believed to result in considerable destruction of adherent sympathetic nerve fibres which may effectively denervate the ischaemic or potentially ischaemic zone. An alternative approach might have been the use of a catheter-guided balloon occlusion of a coronary vessel. Induction of other than proximal occlusions by this technique is, however, difficult.

A major disadvantage of the proposed experimental model is the need for anaesthesia. Anaesthesia may suppress many arrhythmogenic factors, including neurogenic influences, catecholamine release, metabolic changes, as well as having direct membrane effects on the heart. The use of pentobarbitone in these studies may be criticised in view of its suppression of certain reflex effects and its action in inhibiting some degree of adipose tissue lipolysis. The dosage used is, however, standardised for each animal.

Given this basic experimental model of sequential regional myocardial ischaemia in the open-chest dog, studies have been designed to allow for the combined measurement of myocardial metabolic and electrophysiological changes and when indicated, regional myocardial blood flow in order to assess the relative effects of substrate manipulation on each of these variables. If the supposition is correct that metabolic manipulation influences the electrophysiological properties of ischaemic myocardium, then certain metabolic and electrical changes might be expected to follow pari passu.

The specific electrophysiological techniques adopted and the reasoning behind their development are described below. Similarly, problems inherent in metabolic sampling and analysis of regional blood flow are outlined in the critiques of methods.

A description will be given of the development of three major electrophysiological techniques to be used in association with determination of regional myocardial blood flow and metabolic gradients. In keeping with the statement of the major hypothesis of this thesis and its corollary outlined above, studies will be demonstrated

of the natural history of electrophysiological changes during ischaemia which relate to early arrhythmogenesis, control studies of the reproducibility of electrophysiological changes during successive coronary occlusions, and studies of the effects of substrate manipulation following either elevation of arterial glucose levels or reduction of arterial free fatty acid levels by antilipolytic therapy.

# 7. MATERIAL AND METHODS

## MATERIAL

Experimental studies were performed in mongrel dogs, mainly sheepdogs of either sex ranging in weight from 11.5 to 28 Kg. All dogs were fasted for 16 - 20 hours before the start of the experiment. The physical condition of the dogs varied considerably, in that some appeared well fed, whereas others had little subcutaneous fat. All dogs were kept at the Veterinary Research field station for several days prior to laboratory use.

## METHODS

### Anaesthesia

All experiments were performed under pentobarbitone anaesthesia. Induction was performed by initial intravenous injection of 25 - 35 mg/Kg pentobarbitone, this being followed by continuous intravenous infusion of 3 mg/Kg/hour. When necessary, particularly in animals with considerable adipose tissue, additional bolus injections of 30 mg or 60 mg pentobarbitone were given. No metabolic or electrophysiological measurements were performed within 30 minutes of such a bolus injection.

Following intubation ventilation was performed on room air using a volume-cycled Harvard respirator with positive end-expiratory pressure.

Acid-base balance was checked in most studies by determination of arterial pH, $PO_2$ and $PCO_2$, although initial adjustment of ventilation was not found necessary in any study.

A continuous slow intravenous infusion of saline (5 ml/min) was performed through an external jugular vein to allow for insensible fluid loss during the course of the experiment. Body temperature

was maintained above 37°C by means of a thermal blanket when necessary, temperatures being monitored either by rectal thermometer or by a thermistor catheter (Swan Ganz thermodilution catheter) positioned in the pulmonary artery. Pyrexia was managed on two occasions by infusion of cold saline or temporary insertion of ice in the thoracic cavity.

Surgical Procedures

Cannulae were inserted into either or both femoral arteries and femoral veins and in some studies additional catheterisation of external jugular veins and left carotid artery were performed after cut-down.

The femoral venous route were used for intravenous infusion of anaesthetic, drugs, substrates or saline. The external jugular route was used for saline infusion, insertion of thermodilution catheter or atrial or ventricular pacing catheters (No. 7, Zucker).

Arterial pressure was monitored from one femoral line using a Statham P 23db pressure transducer linked to a Devices recorder and blood sampling for biochemical estimation was performed from the second femoral artery. In experiments involving determination of regional myocardial blood flow by the radio-activity labelled microsphere technique the second femoral arterial cannula was used for withdrawal of a reference arterial blood sample.

A 7F Millar catheter-tip pressure transducer was inserted via the left carotid artery in experiments requiring left ventricular pressure or dP/dt measurements.

In studies requiring vagal stimulation the left vagus nerve was dissected free and inserted in a purpose-built sheathed stimulation electrode holder.

A left thoracotomy was performed in all dogs via the fourth or fifth intercostal space. Ties were used in earlier experiments, but diathermy in later studies. The chest was opened with rib retractors and ventilation slightly increased. The pericardium was incised and the heart suspended in a pericardial cradle. Good exposure of the left anterior descending coronary artery, anterior surfaces of the left and right ventricle, left atrium and pulmonary artery thus were obtained.

A typical experimental preparation is shown in Fig. 4.

The left anterior descending coronary artery was dissected free, either proximally or distally to its major diagonal branch and a fine thread passed around the vessel. The exact site varied according to the requirement of the study. A meningeal clip was applied to the vessel to produce regional myocardial ischaemia which became visibly apparent within seconds by the appearance of a zone of cyanosis, often with localised hypokinesia or dyskinesia. Ischaemia was maintained for periods of 5, 10, 15 or over 30 minutes (vide infra) according to the study. Reperfusion was accomplished by removal of the coronary occlusion clip.

Metabolic gradients across the myocardium were determined in some dogs by catheterisation of the coronary sinus, either under fluoroscopic control or direct digital manipulation, and biochemical determinations on arterial and coronary sinus blood withdrawn simultaneously. Alternatively, regional myocardial metabolic gradients were determined following cannulation of a local vein draining from the ischaemic or potentially ischaemic zone of myocardium, again by simultaneous arterial and venous sampling. A vein running along the inner side of the diagonal branch or main left anterior descending coronary artery

FIG. 4. Experimental open chest preparation. The heart is shown suspended in a pericardial cradle. Note the thread around the diagonal branch of the left anterior descending coronary artery, the cannula inserted into a local vein, draining the area supplied by this branch, and the left atrial cannula for radio-active microsphere injections

to the ischaemic area was chosen for cannulation. A 19G needle bent to $90^{o}$ close to its tip was then inserted into the vein, quickly withdrawn and replaced by a fine bevelled-tipped cannula flushed with heparinised saline to prevent clotting. The local venous cannula was then sutured to the surface of the heart to prevent dislodgement. Frequent flushing was necessary to prevent blockage.

In studies requiring left atrial microsphere injection a fine cannula was positioned through a stab incision in the left atrium by a purse-string suture. Fine cannulae were further secured by taping to the rib retractors. Injections, flushing and withdrawal were performed by means of three-way taps.

Needle electrodes were inserted into all four limbs for surface electrocardiographic recordings.

Bipolar atrial pacing electrodes were sutured to the left atrial appendage in all studies.

## Biochemical procedures

Biochemical analyses were performed on blood sampled from femoral artery, coronary sinus or from the local vein draining the ischaemic or potentially ischaemic area. Samples were taken for plasma glucose, free fatty acid (FFA), glycerol, lactate, sodium and potassium estimations (see protocols). Monitoring of arterial pH, $PO_2$ and $PCO_2$ was performed at regular intervals.

Biochemical analyses:-

a) Glucose

Glucose was estimated by the glucose oxidase technique, using a Technicon autoanalyser. The coefficient of variation of the technique was $\pm$ 5%.

Early studies revealed spuriously high glucose levels in some local venous blood samples with slight haemolysis. This was attributed to release of glutathione which then interfered with estimation. Subsequent data was analysed using an automated hexokinase technique (Technicon).

b) FFA

Plasma FFA levels were measured according to Dole's method as modified by Trout, Estes and Friedberg (1960). The final titration was carried out with N/40 NaOH from a microburette using phenol red dissolved in ethyl alcohol as an indicator. This modification provides a single phase titration system making the determination of the end-point more accurate than in Dole's original method (1956).

c) Glycerol

Analysis was performed by the method of Chernick (1969). The coefficient of variation was 3%.

d) Lactate

Analysis was performed by the enzymatic method of Hohorst. Earlier analyses were performed after immediate deproteinisation of 1 ml of whole blood in perchloric acid. Later studies were performed after immediate centrifugation of 200 µl of whole blood, separation of plasma and subsequent plasma extraction. The coefficient of the assay was 6%.

e) Sodium and Potassium

Determinations were made by flame photometry.

f) Blood gases

Blood gas analysis was performed on an I.L. 313 semiautomatic blood gas analyser.

Regional myocardial blood flow determinations

Regional myocardial blood flow was determined by the injection of radio-actively labelled microspheres through a left atrial cannula and subsequent analysis of radio-activity in myocardial tissue slices (Utley et al, 1974).

Before injection $1.5 \times 10^6$ 15 μ microspheres (3M, Riker Laboratories, Loughborough, UK) labelled with $^{141}Ce$, $^{85}Sr$, $^{57}Co$, $^{113}Sn$ or $^{95}Nb$ were sonicated in 10% dextrose for 10 minutes to dispel aggregates and drawn into a syringe containing 0.1 ml 5% Tween 30. The suspension was diluted with 10% dextran to a final concentration of Tween 80 of less than 0.5%. Microspheres were injected at a predetermined time with respect to coronary occlusion (see protocols). Femoral arterial blood was withdrawn into a heparinised syringe at approximately 5 ml/min, commencing 30 seconds before injection and continuing for 1½ minutes after injection for use as reference blood sample. Injections of up to four differently labelled radio-active microspheres were given in any one animal at various times during the study.

After each experiment the heart was examined, the free wall of the left ventricle removed and superficial fat and blood vessels removed. Sixteen to 18 full thickness tissue samples, approximately 1 $cm^2$ were obtained with reference to an anatomical diagram of the heart drawn during the experiment. Samples were divided into endocardial and epicardial segments and weighed separately. Radio-activity counts on each sample were determined using a "Wallac" gamma counter with simultaneous counting from two channels. Band widths were set appropriately for each isotope. Regional myocardial blood flow was calculated using the formula:

Regional myocardial blood flow (validation of technique)

In view of the possibility that errors in regional myocardial blood flow determinations might arise from the use of microspheres labelled with isotopes of differing density and hence differing flow distribution, comparability studies were performed in our laboratory (Dr. R.A. Riemersma, unpublished) by simultaneous injection of 15 μ microspheres labelled with $Co^{57}$, $Ru^{103}$ and $Sc^{46}$ respectively. $1.5 \times 10^6$ spheres of each isotopic labelling were injected 5 minutes after occlusion of the left anterior descending coronary artery to enable measurements over a wide range of coronary flow. In 4 studies no significant differences could be found between values of epicardial flow, endocardial flow or endocardial/epicardial blood flow ratios in either ischaemic or non-ischaemic myocardium as determined by the 3 isotopes. A highly significant correlation was found for values of regional blood flow as determined by the separate isotopes, e.g. for $Co^{57}$ and $Ru^{103}$ over a wide range of blood flows, $r = 0.992$, $P < 0.001$, sample number = 144. No radioactivity above background was detected in 500 ml blood collected 30 minutes after microsphere injection excluding the possibility of a carry-over effect. Nevertheless, in all studies the order of microsphere injections was randomised. To minimise errors which might arise from reference blood sampling, withdrawals of arterial blood were performed by constant withdrawal pump and accurately timed with a stop-watch.

control studies requiring infusion of the non-metabolised sugar mannitol, infusions at the same rate and concentration were given

to achieve isosmolar concentrations of sugar.

b) Manipulation of plasma FFA

Direct infusion of FFA to elevate plasma levels is not possible due to induction of marked haemolysis, platelet thrombi and toxic effects of FFA-micelles unbound to albumin. Profound hypotension is induced (Riemersma, 1973). An alternative technique is elevation of FFA using triglyceride-heparin (Kurien et al, 1969; Opie et al, 1971; Kjekshus and Mjøs, 1972; Kostis et al, 1973), but associated release of lipoprotein lipase into plasma has been shown in our laboratory to lead to gross overestimates of true plasma FFA levels following plasma extraction, due to in vitro lipolysis (Riemersma et al, 1977). In the absence of a technique for FFA estimation following immediate blood extraction (under development at this time) this method was not considered sufficiently precise.

An indirect method was employed therefore in comparing conditions of elevation of plasma FFA as a result of catecholamine induced stimulation of lipolysis with conditions of inhibition of stimulated lipolysis by an antilipolytic agent. Stimulation of lipolysis was achieved by infusion of isoprenaline 0.1 µg/kg/min over a period of 20 to 30 minutes. Pilot studies showed that although the inotropic effect of isoprenaline was apparent within two minutes, the metabolic response was delayed for 15 to 20 minutes.

Inhibition of catechol-induced stimulation of adipose tissue lipolysis was achieved by infusion of either nicotinic acid 0.5 to 1.0 $mg.Kg^{-1}.min^{-1}$ or its derivative, 5-fluoronicotinic acid, together with isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$. Addition of nicotinic acid has been shown to have no additional haemodynamic effect (Kjekshus and Mjøs, 1973), whilst lowering plasma FFA to within normal levels.

## Statistics

Unless otherwise stated, statistical significances were judged by paired t-testing using a programmable Hewlett-Packard desk-top computer.

## Critique

The need for and potential disadvantages of an open-chest anaesthetised preparation have been discussed. Care was taken to ensure an even and adequate level of anaesthesia as electrophysiological variables, particularly ventricular vulnerability, were found to be extremely sensitive to small changes in depth of anaesthesia. Similarly, at lighter depths of anaesthesia on two occasions manipulation of a three-way tap for blood sampling purposes was found to influence the VPB threshold. Multiple electrophysiological determinations were necessary prior to occlusion of the coronary vessel to ensure reproducibility of data uninfluenced by inadequate anaesthesia. The use of alternative anaesthesia, such as chloralose, has been advocated rather than pentobarbitone for some electrophysiological studies in view, for example, of preservation of certain vagal effects. A consistency of use of anaesthesia between studies was considered of great importance, however, and the same anaesthesia used in all studies.

Variations in acid-base balance are known to have both electrophysiological and metabolic effects. Metabolic acidosis may reduce and metabolic alkalosis increase the ventricular fibrillation threshold in the non-ischaemic dog heart (Gerst et al, 1966). Electrophysiological or metabolic effects resulting from transient variations in acid-base balance due to alterations

in ventilation or other manipulations cannot be excluded therefore in this experimental model, despite intermittent blood gas monitoring. Neither respiratory acidosis nor respiratory alkalosis have been shown to affect the ventricular fibrillation threshold in dogs, however (Gerst et al, 1966). Hyperventilation would not therefore be expected to greatly influence electrophysiological data. Hypoventilation could be a factor by virtue of potential hypoxia, rather than hypercapnia. Similarly, local cooling of the heart, either as a result of hypothermia or direct exposure to air following thoracotomy, can have marked electrophysiological effects by prolongation of action potential duration, refractory period and conduction times (Hoffman and Cranefield, 1960). Effects on arrhythmogenesis are more marked if a temperature gradient is generated across the myocardium (Mouritzen and Anderson, 1965; Wallace and Mignone, 1966), and are related to the speed of generation of such a gradient. Such temperature differentials may exist between epicardium and endocardium in the experimental model and even be enhanced during ischaemia when reduced coronary flow diminishes heat transfer. Some degree of temperature control was achieved with the use of a heating blanket, but additional precautions, such as filling the thoracic cavity with an insulating layer of plastic foam, were not performed. A further general consideration is maintenance of a stable haemodynamic status. Considerable loss of plasma volume can follow insensible fluid loss by ventilation unless replacement infusion is administered. An arbitrary slow intravenous saline infusion was used to counter this objection, but may not have been adequate in some dogs. Other factors influencing blood loss, such as progressive haemorrhage from small vessels, have not been quantitated.

The validity of the technique adopted of assessment of metabolic changes during ischaemia from arterial-local venous differences may be questioned. Although arterial blood undoubtedly perfuses the ischaemic region via collateral branches from non-occluded vessels, the blood sampled from the local vein draining from the ischaemic zone probably represents a mixture of venous blood from ischaemic regions of varying severity and from non-ischaemic myocardium. The degree of venous admixture was not determined, although this might have been possible by radio-active tracer techniques. Further, the greater the degree of ischaemia, the less the relative contribution to venous effluent flow from the ischaemic region and the greater the likely dilution with venous effluent blood from non-ischaemic regions. In addition local regional redistribution of blood flow during the first few minutes after coronary occlusion due to release of vasoactive metabolites may influence the pattern of venous admixture, irrespective of the degree of severity of ischaemia. Nevertheless, striking metabolic changes can be detected from biochemical analysis of local venous blood during ischaemia, compared with values obtained before occlusion which must relate to altered myocardial metabolism. In addition each study in each dog serves as its own control. Simultaneous comparisons of arterial-local venous differences of different substrates may be considered independent of changes in regional flow distribution and indicative of substrate exchange. Similarly, comparison of arterial-local venous differences of substrates between successive occlusions may be permissible if no change in regional myocardial blood flow at these times is demonstrated. A stricter comparison

of ischaemic arterial-local venous differences of substrates with non-ischaemic values might have been achieved by simultaneous sampling from either the coronary sinus or a local vein draining from a non-ischaemic region of myocardium. Venous admixture effects may still be observed, however, due to some contribution to venous effluent from the ischaemic region. A further problem is that of attainment of steady-state levels of substrate exchange. This is unlikely to occur within the first 10 minutes of ischaemia due to variability in regional flow, substrate metabolism and exchange kinetics. Tissue gradients of metabolites may vary considerably according to the degree of severity of ischaemia. Even if local venous effluent blood is taken to represent true "ischaemic" effluent, it probably derives only from those areas within the ischaemic region which are supplied by collateral perfusion, probably mainly epicardial layers which have a relatively higher blood flow (Regan et al, 1972; Becker et al, 1973) and which may account for only a fraction of cells in the ischaemic region. An alternative technique which might have been adopted therefore is the direct analysis of tissue metabolic changes by biopsy techniques. Such procedures are, however, invasive and do not allow for sequential analyses in the same preparation. In addition the time required for biopsy sampling, even with a high speed drill, is considerably longer than that required for accurate analysis of such labile metabolites as high energy phosphates. In view of the variability of regional ischaemic response in the dog, enormous numbers of experiments would be required in this preparation to assess the relationship between electrophysiological and tissue metabolic

events. A more promising and alternative non-invasive approach under development in our laboratory is that of an assessment of the regional redox state of the myocardium by fluorescent photographic techniques, but this is as yet not operational.

Even small alterations in regional myocardial blood flow distribution during ischaemia may alter the cellular response and hence any electrophysiological effect. The technique of assessment of regional myocardial blood flow using radio-actively labelled microsphere injections has the advantages of providing reasonably accurate estimates of regional flow in multiple tissue biopsies from both endocardium and epicardium. Washout techniques such as with $^{133}$Xenon give only measures of averaged coronary flow in a region and can suggest a misleading uniformity of flow and cannot detect endocardial-epicardial flow ratios. Similarly, regional information is not gained by the hydrogen saturation method. Certain criticisms may be applied to the microsphere technique, however (Buckberg et al, 1972). Because of the random distribution of spheres injected into the arterial circulation, precision of flow determination is related to the number of spheres lodged in each tissue sample. The number of 400 spheres per sample has been taken as a limiting quantity. This value determines the number of spheres injected. In severe ischaemia with extremely low flows, it is likely that larger numbers of spheres would have been required to be injected than used in these studies. All studies presented were performed in more moderate ischaemia or in normal myocardium and sufficient sphere density obtained. Other potential problems could arise from inadequate mixing of spheres, plasma streaming or preferential sphere distribution relating to sphere diameter with failure of trapping. The 15 μ sphere diameter

was chosen as smaller spheres show a greater incidence of failure of trapping in the vascular bed and larger spheres may show preferential regional flow distribution. No haemodynamic disturbances have been reported in the literature following microsphere injections and indeed recent studies in this laboratory have shown no effect on left ventricular pressure of dP/dt of injection of 12 x $10^6$ spheres.

A knowledge of both regional flow and arterial-venous differences of a substrate or metabolite permits an analysis of absolute substrate exchange. Arteriovenous shunting may not be detected, however, by microspheres and lead to spurious estimates of substrate exchange. In addition during ischaemia the degree of venous admixture between normal and ischaemic venous effluent blood cannot be assumed from regional arteriolar or capillary flow distribution. No calculation is made of substrate exchange therefore in this thesis, although it is suggested that alterations of arterial-local venous differences during ischaemia in the absence of a detectable change in regional flow distribution are metabolically meaningful.

## 8. ELECTROPHYSIOLOGICAL PROCEDURES

The electrophysiological techniques adopted for use in the open-chest anaesthetised dog preparation have been devised to allow for:-

a) an analysis of the frequency of ventricular arrhythmias or fibrillation during acute myocardial ischaemia.

b) an assessment of quantitative changes in the vulnerability of the ventricle to the development of arrhythmias or ventricular fibrillation during acute myocardial ischaemia.

c) an assessment of the underlying changes in the electrophysiological properties of the ischaemic myocardium that predispose to or result in enhanced vulnerability to arrhythmias or overt arrhythmogenesis.

d) an analysis of the relationship between these changes and metabolic gradients in the heart and alterations in regional myocardial blood flow.

e) an analysis of the influence of substrate manipulation on these changes.

Ventricular premature beats or fibrillation are easily detected from the surface or epicardial electrogram and a method of subsequent computer analysis from tape recorded signals has been utilised.

In view of the complexities of electrophysiological events during acute myocardial ischaemia, and of the uncertain relationship between individual variables and arrhythmogenesis, it was considered that some studies should examine an index of ventricular vulnerability. The measurement of ventricular fibrillation threshold has been commonly used as such an index (vide infra) and initial studies will be presented using this technique. The reasons for its unsuitability for the requirements of this thesis and the development of the alternative technique of ventricular premature beat threshold measurement will be shown. Considerable advantages arise from the assessment of ventricular vulnerability in the absence of overt spontaneous arrhythmias which may themselves produce secondary haemodynamic or electrophysiological effects. Alternative indices of ventricular vulnerability, including assessment of "vulnerable zones" or strength-interval curves for induction of premature beats, were examined in some feasibility studies, but the time required for suitable data collection greatly exceeded the duration of the period of enhanced ventricular vulnerability under examination. All studies will be seen to be performed at constant heart rate by overdrive atrial pacing in view of known rate dependent effects.

The choice of local or regional electrophysiological variables for study was determined by their likely importance or relation to metabolic influences on arrhythmogenesis. Many workers have used the index of epicardial ST-segment elevation as a measure of ischaemic injury and in the assessment of metabolic interventions (see Section 5). Such an index is unreliable, however, in the presence of conduction delays of an order required to induce overt arrhythmias due to local depolarisations arising within the period of the recorded ST-segment. ST-segment determinations will be restricted therefore to milder or more moderately ischaemic preparations. Likely mechanisms of arrhythmogenesis include re-entrant excitation and enhanced automaticity (Section 2). Variables believed to predispose to re-entrant activity and which are also subject to metabolic influences include inhomogeneity of refractoriness, conduction delays and intermittent conduction blocks (Wit et al, 1974). The very existence of inhomogeneity of refractoriness precludes determinations of global ventricular refractoriness. An experimental model will be presented therefore which allows sequential determinations of epicardial ventricular refractoriness in both normal and central and border areas of ischaemic myocardium. It was considered that inhomogeneity might be greater between widely separated groups of cells than between closely adjacent groups of cells within the ischaemic zone. Associated variations in diastolic excitability threshold are an important determinant of refractoriness during ischaemia, but the need for rapid sequential data collection has not allowed combined measurement of this variable with changes in refractoriness. Recording of conduction delay during ischaemia is possible by analysis

of the time delay between local depolarisations detected by adjacent surface unipolar or bipolar electrograms. Such analyses are difficult following fractionation or partial fractionation of the wavefront which may lead to a considerable range of epicardial activation times beneath a surface epicardial electrode. An experimental model will be presented therefore which permits determination of conduction delays with respect to recordings from an epicardial micro-electrode recording from a single cell or small group of cells. Action potential recording also permits detection of local cellular conduction abnormalities which are only apparent as fine repolarisation abnormalities on the surface electrogram. A complete analysis of wavefront excitation patterns, although ideal, is beyond the scope of current electrophysiological technology and thus necessitates such inferential analysis. Additional reasons for the development of action potential recording have been the possibility of demonstrating slow diastolic depolarisations which could relate to enhanced automatic activity and the known associations between changes in action potential morphology and alteraions in cardiac metabolism, in particular the close relation under some conditions between the duration of the plateau phase of the action potential and cellular ATP content (McDonald and McLeod, 1973; Cheneval et al, 1972). Definitive studies investigating automaticity have not been performed. Feasibility studies using the techniques of induction of complete heart block by formalin injection or vagal stimulation in order to assess ventricular escape times proved difficult to interpret during ischaemia. Reproducibility of data under given conditions was poor, ventricular fibrillation common and rapid sequential

measurements not possible. In addition there is little evidence that enhanced automaticity plays an important role in the genesis of early arrhythmias.

Three specific electrophysiological techniques have been developed and used therefore and each will be discussed separately:-

a) Determination of ventricular premature beat thresholds

b) Determination of regional functional refractory periods

c) Combined determination of intracellular and extracellular potential changes using the floating micro-electrode technique

The developmental processes, together with some results of initial pilot studies, will be described.

## (a) VENTRICULAR PREMATURE BEAT THRESHOLD STUDIES

The initial intention was to utilise a system of ventricular fibrillation threshold (VFT) determination as an index of ventricular vulnerability (Han, 1969; Burgess et al, 1971; Bloor et al, 1975).

A fibrillation system was designed and and constructed, in conjunction with the Medical Physics Department of the Royal Infirmary (Dr. J.M.M. Neilson, Mr. I. Williamson).

A block diagram of this system is shown in Fig. 5, and a photograph of the completed devices shown in Fig. 6.

Bipolar electrodes embedded in plastic (1.5 mm diameter and 4 mm apart) were sutured to the left atrium and left ventricle for atrial pacing and application of fibrillatory pulses respectively.

Atrial pacing stimuli were derived from a pacing unit (isolation transformer) with variable gain driven by the basic control unit. Pacing pulses were 2 ms duration and the pacing rate continuously variable between 100 and 300 beats/min. A pacing trigger output from the control unit permitted triggering of an oscilloscope (Tektronics DM 63) at the atrial pacing rate.

The addition of a variable delay circuit provided a second output from the control unit to the fibrillation current (or constant current source). The fibrillation pulses were 4 msec in duration, and by means of a switch on the control unit, could be applied either (a) singly or (b) in trains at 10 ms intervals during a burst of 250 ms duration. The specifications of the constant current source allowed for generation of currents of 0 - 50 mA.

The delay between the pacing pulse and start of the fibrillation stimulus was continuously variable between 200 and 400 ms.

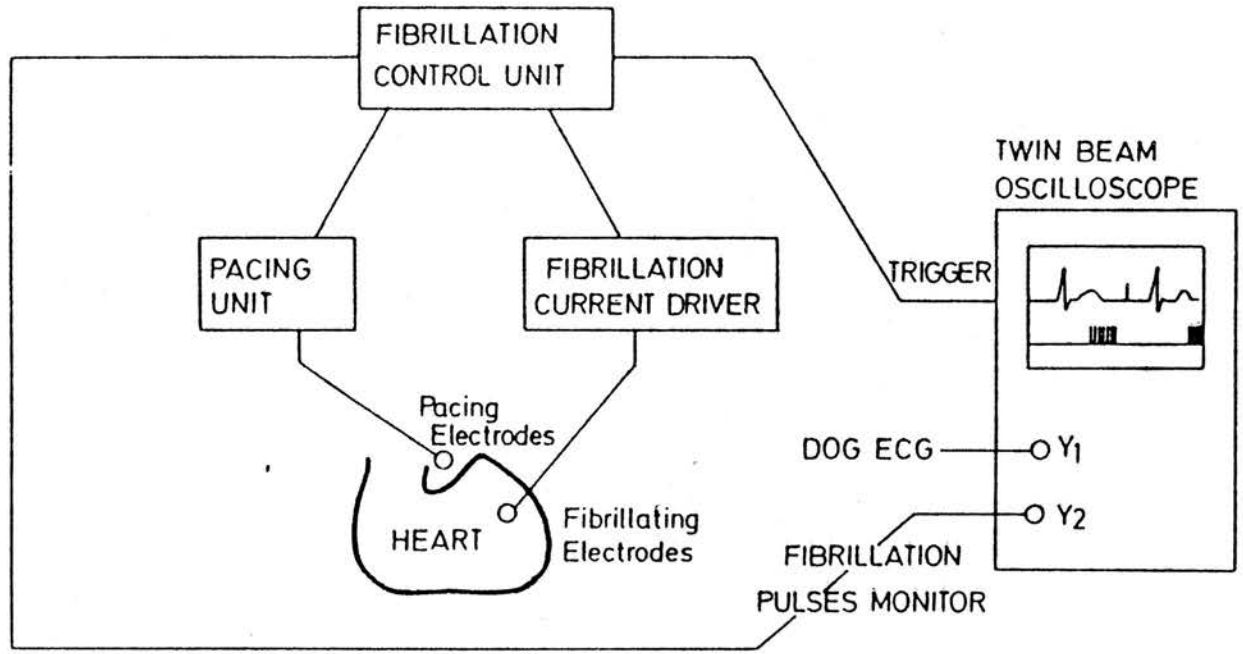


FIG. 5. Experimental arrangement for ventricular fibrillation threshold determination

A further monitoring output from the control unit permitted concurrent display of the dog's electrocardiogram and the fibrillation stimuli so that the required delay could be set up.

The facility for a single, rather than a train of fibrillatory pulses, was designed to permit refractory period determinations and also quantitative analysis of the vulnerable zone.

In view of the possibility of excessively high electrical resistance developing between the animal's heart and the fibrillation electrodes, an audio-alarm was included in the current driver. Consequently, a high-pitched tone was produced if the necessary fibrillatory current was not attained. In practice the alarm was initiated most commonly by intermittent electrode contact with the heart or lead breakage. A battery level meter was also included.

Pilot experiments with VF threshold determination

Two pilot experiments utilising this system were unsatisfactory. Overdrive atrial pacing at 175 beats/minute was employed using the open-chest dog preparation. Fibrillatory electrodes were sutured to the anterior surface of the left ventricular epicardium and the train of current pulses positioned so as to commence at the peak of the T wave of lead II of the electrocardiogram over the so-called "vulnerable" zone. Stimulation was commenced at just suprathreshold ( < 500 μA) and increased by 500 μA increments, allowing at least 12 beats between test stimuli. Under these conditions fibrillation resulted between 1 and 2 mA of stimulation current. Sequential VFT determinations resulted in VF at just suprathreshold levels of current.

A higher VFT could be obtained in three further studies by commencing the train of pulses immediately after onset of ventricular


FIG. 6. Purpose-built equipment for determination of ventricular fibrillation threshold and used in determination of ventricular premature beat thresholds. The control unit (centre) is shown, together with the pacing unit (above) and constant current source (below)

depolarisation, as the very low VFT's obtained in the earlier studies probably related in part to the determination of the VFT of a ventricular premature beat induced earlier in the train of pulses (Burgess et al, 1971). Defibrillations were performed utilising a DC defibrillator capable of delivering 15 or 20 W shocks through internal paddles moistened with saline. Repeated determinations of VFT in these studies gave values in the normal non-ischaemic heart of 8.7 $\pm$ 3.4 mA (n = 9), 3.2 $\pm$ 0.4 mA (n = 13) and 5.5 $\pm$ 2.3 mA (n = 9). The range, however, was considerable, viz. 6.1 to 15.4 mA, 2.5 to 3.8 mA and 2.2 to 8.0 mA respectively. At least 20 minutes was allowed following each defibrillation for recovery of the myocardium. Provided defibrillation was performed within five seconds of fibrillation, blood pressure was unaffected by DC shock.

Coronary occlusion was performed in each animal and VFT's determined after five minutes of ischaemia in successive occlusions. Data was reproducible in two dogs, viz. 2.2 mA, 2.5 mA, 2.25 mA, 2.25 mA in one dog; and 1.6 mA, 2.0 mA, 0.3 mA, 0.3 mA in another. In a third dog, however, data was more variable, viz. 5.4 mA, 1.2 mA, 0.2 mA, 7.5 mA, this variation being again attributable to the determination of VFT of an induced premature beat.

The VFT technique was considered unsuitable for analysis of metabolic intervention studies during ischaemia, for the following reasons:-

1. The need for repeated defibrillation, particularly during ischaemia with probable secondary effects on contractility, blood flow, catecholamine release and myocardial metabolism.

2. The restriction to one determination every 15 minutes, although more frequent determinations have been reported during ischaemia (Lown and Varrier, 1976).

3. The wide range of control data in any individual dog.

4. The low values, in some cases almost suprathreshold of VFT, of control data.

5. The lack of consistently reproducible data during ischaemia.

Pilot experiments with VPB threshold determination

An alternative approach to assessment of ventricular vulnerability by VF threshold determination is to use single or multiple extrasystoles or premature beats as the end-point instead of VF (Thompson and Lown, 1972; Lown et al, 1973; Verrier and Lown, 1976). Such a repetitive extrasystole threshold determination utilising "R/T pulsing" has been shown to give values 2/3 of the value of the VFT reproducibility without actually inducing fibrillation (Matta et al, 1976). Such a system should therefore have the advantage of providing the same information as VFT regarding ventricular vulnerability without the necessity of repeated defibrillation.

A system for determination of ventricular premature beat thresholds (VPBT) was therefore devised and is shown in block diagram form in Fig. 7. Measurement of VPBT requires the positioning of two 'primary' ventricular extrasystoles, such that each arises within the presumed 'vulnerable' period of the preceding beat, in the case of the first pulse within the ventricular vulnerable period of the normal paced supraventricular impulse. A third pulse must then be positioned within the presumed vulnerable period of the second induced VPB and its current strength increased until multiple extrasystoles are produced. Further increase in current strength induces fibrillation and a VFT may be measured.

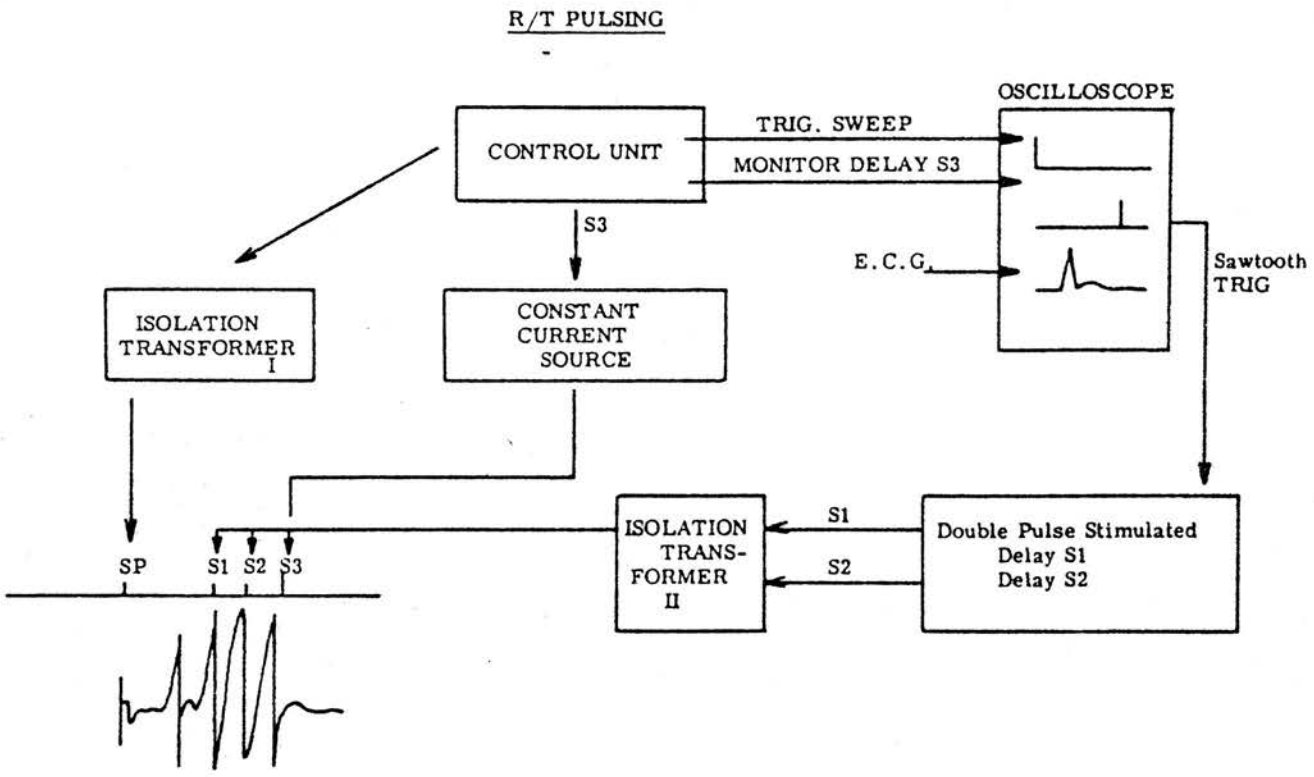


FIG. 7. Diagrammatic representation of the experimental arrangement for VPB threshold determinations

This was achieved by the addition of a purpose built double pulse stimulator with the facility for external triggering (Mr. J. Austin, Department of Physiology, Edinburgh University).

Two pairs of bipolar electrodes were sutured, in close proximity, on the left ventricular surface for application of primary and test pulses respectively, in addition to the left atrial pacing electrodes.

Both the pacing unit and DM 63 storage oscilloscope were triggered by the control unit at the desired pacing frequency (175 beats/minute). The double pulse stimulator, triggered from the sawtooth output from the oscilloscope, was used to apply two timed 2 ms pulses to an isolation transformer and hence to one of the pairs of epicardial electrodes. These pulses were constant voltage, set at twice mid-diastolic threshold and applied at 15 msec beyond the functional refractory period of the preceding beat. The second pulse was set to commence 15 ms beyond the functional refractory period of the VPB induced by the first pulse. By setting the oscilloscope to single sweep, two primary pulses could be produced on demand. The third, or test, pulse was obtained on demand on pressing the "fibrillate" button on the control unit and was passed via the constant current source to the second pair of bipolar electrodes. This pulse was of 4 ms duration and positioned 15 ms after the previously determined functional refractory period of the second induced primary premature beat.

A characteristic series of electrocardiographic traces using this system is shown in Fig. 8. At a current strength of 4 mA the third extrastimulus produces a single ventricular response. On progressively increasing the current strength of the third extra-stimulus, however, a point is reached, in this example at 32 mA, when


FIG. 8. Surface lead electrocardiographic recordings of induction of three repetitive ventricular premature beats. By increasing the current strength of the third extrastimulus to 32 mA a single additional VPB occurs, at 38 mA repetitive extrasystoles and at 45 mA ventricular fibrillation

an additional VPB is induced. On further increasing the current strength of the third extrastimulus multiple VPB's (38 mA) and finally VF (45 mA) result.

An interval of at least 12 cardiac cycles was allowed to elapse between test runs. The VPBT is thus defined as the current strength required to induce one or more additional VPB in two out of three trials. A test sequence was derived therefore in each study commencing at some arbitrary current strength below the VPBT and increasing in increments of 5 mA initially, and increments of 1 mA immediately beneath the VPBT.

To confirm the predicted relationship between VPBT and VFT, both measurements were sequentially determined in two dogs. The values obtained in one of these studies, both before and during a 15 minute period of coronary occlusion, are shown in Fig. 9. The two variables can be seen to follow each other pari passu and the mean ratio $\frac{VPBT}{VFT}$ was 0.67, closely agreeing with Verrier and Lown (1976).

Control data of VPBT in the normal heart was acceptable in three further pilot experiments, viz. 34.3 $\pm$ 8.5 mA (n = 11), 42.2 $\pm$ 1.9 mA (n = 11), 26.1 $\pm$ 2.4 mA (n = 10) (mean $\pm$ S.D.), as was its reproducibility during successive coronary occlusions (see Results).

Reproducible results were not obtained during ischaemia with positioning of the stimulating electrodes on the ischaemic zone. Stimulating electrodes were positioned well away from the ischaemic or potentially ischaemic area therefore.

Satisfactory data could be obtained at one minute intervals using this technique of VPBT determination, both before and during successive 10 minute coronary occlusions.

VF resulted in only one out of 150 test runs and was managed

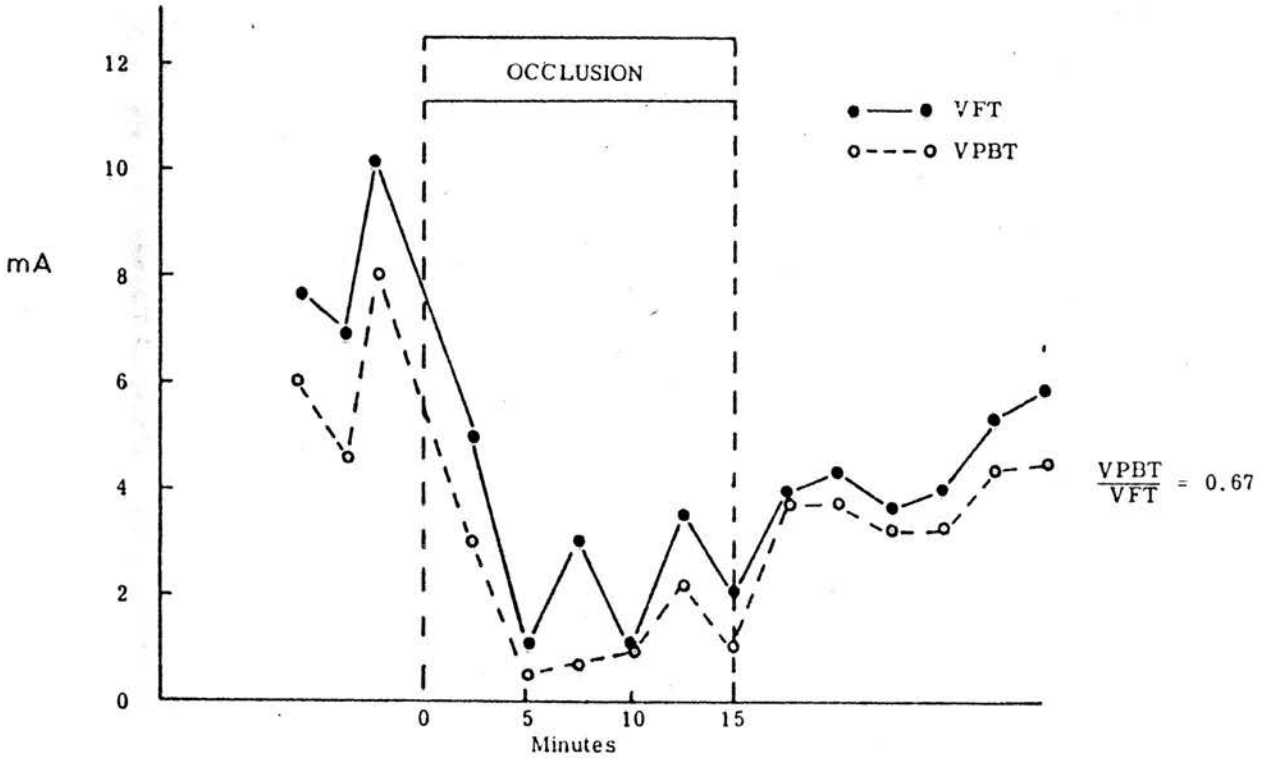


FIG. 9. Comparison of ventricular fibrillation threshold and ventricular premature beat threshold determinations in one dog before, during and after a 15 minute coronary occlusion. VFT determinations were made closely after VPBT determinations. A ratio of 0.67 was obtained between meaned VPBT and VFT determinations

by immediate DC defibrillation.

Data from these studies was recorded on tape and subsequently played back through an arrhythmia computer (Neilson, 1974). A single 1 s square wave output from the oscilloscope over the period of each test sequence was recorded on tape and subsequently fed into the computer, together with the surface electrocardiogram. By this means VPB artifically produced by test stimulation were excluded from analysis. A frequency distribution curve of spontaneous as opposed to artificially induced ectopic beats could then be produced.

Protocols

(i) Studies were performed in 14 dogs to test the effect of intravenous glucose on ventricular vulnerability following acute coronary occlusion.

The experimental protocol adopted is shown in Fig. 10. An initial occlusion (not shown) was performed to determine the approximate extent of ischaemia and to act as a sham occlusion. The coronary occlusion clip was applied just proximal to the bifurcation of the diagonal branch of the LAD or in some dogs on the diagonal branch itself. Three further consecutive 10 minute test occlusions were performed, an interval of at least 30 minutes being allowed to elapse between occlusions to allow for recovery. Acid-base balance between occlusions was maintained by arterial $PO_2$, $PCO_2$ and pH measurements. Measurements of VPBT were made at one minute intervals both before, during and for 10 minutes after the coronary occlusions.

Intravenous infusions were given commencing five minutes prior to occlusion and continuing for 10 minutes after release of the occlusion. Isotonic saline was infused at a rate of 0.85 $ml.min^{-1}$ during the

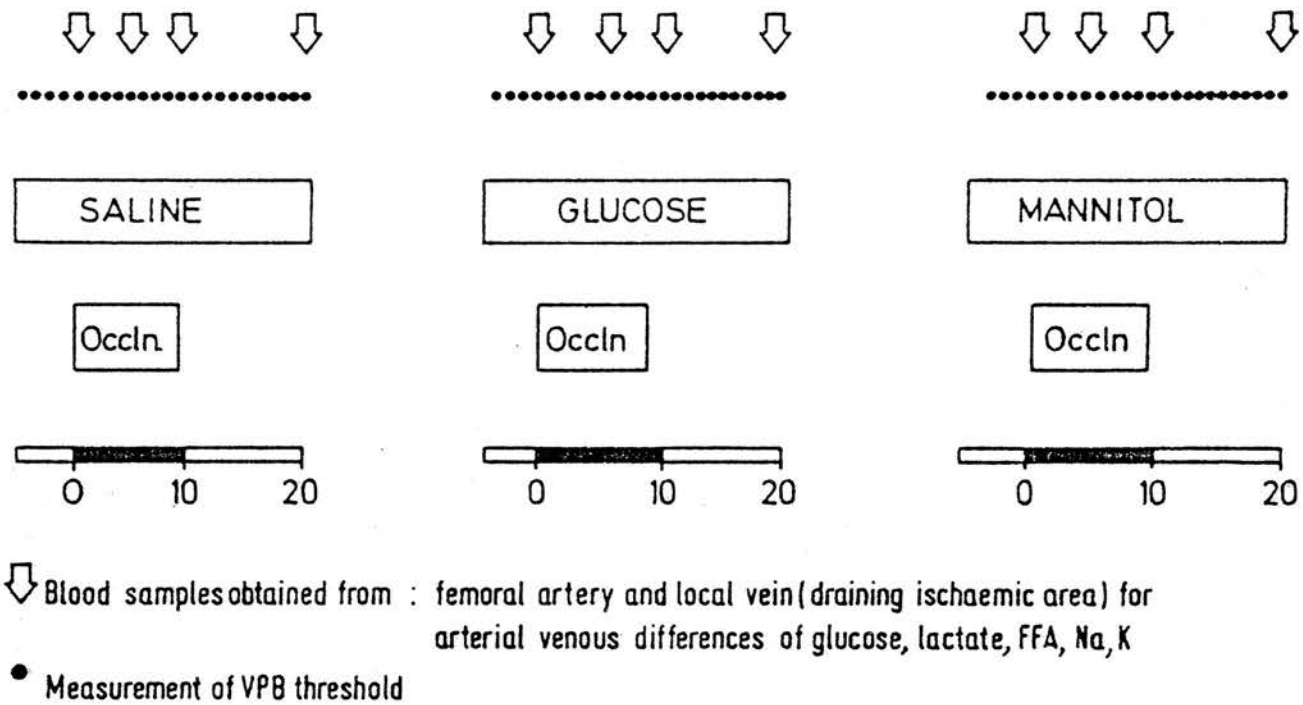


FIG. 10. Experimental protocol adopted in group A dogs. In group B the order of glucose and mannitol infusions were reversed

period of the first occlusion. Animals were then divided into two experimental groups. In group A (8 dogs) glucose was infused during the second occlusion at a rate of 37.5 $mg.Kg^{-1}.min^{-1}$ for the first five minutes and 17.5 $mg.Kg^{-1}.min^{-1}$ subsequently in order approximately to double blood levels. Mannitol at the same concentration was infused over the period of the third occlusion (6 dogs). In group B (6 dogs) the order of glucose and mannitol infusions was reversed.

Blood sampling was performed from the femoral artery and the local vein draining the ischaemic area for biochemical estimations of glucose, lactate, sodium, potassium and FFA at 0, 5, 10 and 20 minutes after occlusion.

(ii) The effect of inhibition of isoprenaline stimulated lipolysis on ventricular vulnerability (VPBT) was examined in seven dogs.

Following sham occlusion three consecutive test occlusions of 10 minutes duration were performed as above. Isotonic saline was infused at a rate of 0.85 ml/min over the period of the first occlusion. Isoprenaline infusion was commenced 20 minutes preceding the second occlusion and continued throughout the second occlusion at a rate of 0.1 $\mu g.Kg^{-1}.min^{-1}$ (3 dogs) or 0.01 $\mu g.Kg^{-1}.min^{-1}$ (4 dogs). The lower infusion rate was given to test the possibility that plasma FFA might be elevated without associated inotropic effect of catechol stimulation.

Measurements of VPBT were made at one minute intervals and blood sampling was performed at 0, 5, 10 and 20 minutes after occlusion.

Over the period of the third occlusion the antilipolytic agent SAB 515 (5-fluoronicotinic acid) was infused at a rate of 5 $mg.min^{-1}$, together with isoprenaline at rates of 0.1 or 0.01 $\mu g.Kg^{-1}.min^{-1}$ (7 dogs). Measurements of VPBT and blood sampling were again carried out as above.

Critique

Assessment of ventricular vulnerability by VPB threshold determination has the advantage over VF threshold determinations in that repeated defibrillation becomes unnecessary. Large current strengths of up to 50 mA are required, however, which could produce local tissue damage at the site of stimulation. Local catecholamine release at the site of application of a train of gated extrastimuli has been demonstrated during VF threshold determinations (Brady et al, 1960; Moe, 1978). A similar effect could modulate the electrophysiological response to test extrastimuli. Reproducibility of VPB threshold determinations compared favourably with those of VF threshold determinations of other workers (Han, 1969; Burgess et al, 1971; Bloor et al, 1975). The use of a single test stimulus, rather than a train, eliminated the possibility of measurement of vulnerability of an additionally induced premature beat. Critical positioning of both primary and test impulses with respect to the functional refractory periods of the preceding beats was necessary however. Readjustment of positioning of test impulses had to be carried out prior to each coronary occlusion to ensure the position of maximum vulnerability. It is assumed that refractory period in the normal zone does not change significantly over a 10 minute occlusion period as time is not available to continually scan the vulnerable period. In practice, no changes were observed in normal zone refractoriness. Similarly, it is assumed that complete electrophysiological recovery follows 12 cardiac cycles after a test impulse. This may not hold in ischaemic tissue to automatic activity or membrane depolarisation. Care was taken to ensure that the site of stimulation was well away

from the ischaemic zone. A slight shortening in refractoriness in the test zone could spuriously elevate VPB threshold by shifting the vulnerable zone towards the preceding beat. Evidence suggests, however, that the vulnerable zone is expanded rather than shifted during ischaemia (Lazzara, 1978).

Elevation in VPB threshold on reperfusion could have been artefactual due to a prolongation in action potential duration or refractoriness observed in later studies, even in normal zone tissue by the mechanism of shifting the vulnerable zone away from the preceding beat.

Experimental control was achieved by use of two consecutive test occlusions following an initial sham occlusion, and with order reversal of glucose and mannitol infusions during test occlusions. A metabolic carry-over effect of glucose infusion in terms of insulin release might have influenced a subsequent occlusion. A similar carry-over effect of mannitol by virtue of its longer plasma half-life on glucose may have occurred. The absence of change in osmolality and the lack of significant changes in plasma glucose levels from control during second test mannitol infusions would suggest minimal influence of these effects.

Care in interpretation of metabolic data from arteriovenous differences of substrates is required. Local venous admixture effects of blood draining from normal tissue via collateral vessels and blood draining from ischaemic tissue occur. In the absence of flow data, interpretation can only be based upon directional changes in arterio-venous differences. Even determination of regional blood flow does not demonstrate the relative contribution to venous effluent of flow in ischaemic and non-ischaemic areas. Substrate uptake or release cannot be determined during ischaemia in this experimental model.

(b) REGIONAL VENTRICULAR REFRACTORY PERIOD STUDIES

An alternative approach to that of examining the vulnerability of the whole ventricle to development of arrhythmias is to examine changes in specific electrophysiological variables believed to be of importance in the pathogenesis of ventricular arrhythmias. An important mechanism is thought to be that of re-entrant excitation (Wallace and Mignone, 1966; Cranefield and Hoffman, 1971; Wit et al, 1974). This mechanism is of particular importance in early as opposed to later onset arrhythmias (Lazzara, 1978).

Predisposing factors to re-entrant activity include:-

a) Conduction delay

b) Unidirectional conduction block

c) Inhomogeneity of ventricular refractoriness (Wit et al, 1974)

Inhomogeneity of refractoriness could predispose to re-entrant excitation by promoting conduction of excitation from an area of prolonged refractoriness to an area of shortened refractoriness or by permitting abnormal conduction of a ventricular premature beat from some other source.

An experimental model has been devised therefore to allow for determination of regional ventricular functional refractory periods (FRP) in normal and ischaemic myocardium. In view of likely inhomogeneity between central and border ischaemic areas, the model was designed to permit FRP determinations in putative central and border zones of ischaemic myocardium as well as in control non-ischaemic zones.

A schematic representation of the experimental system is shown in Fig. 11. Following suspension of the heart in a pericardial

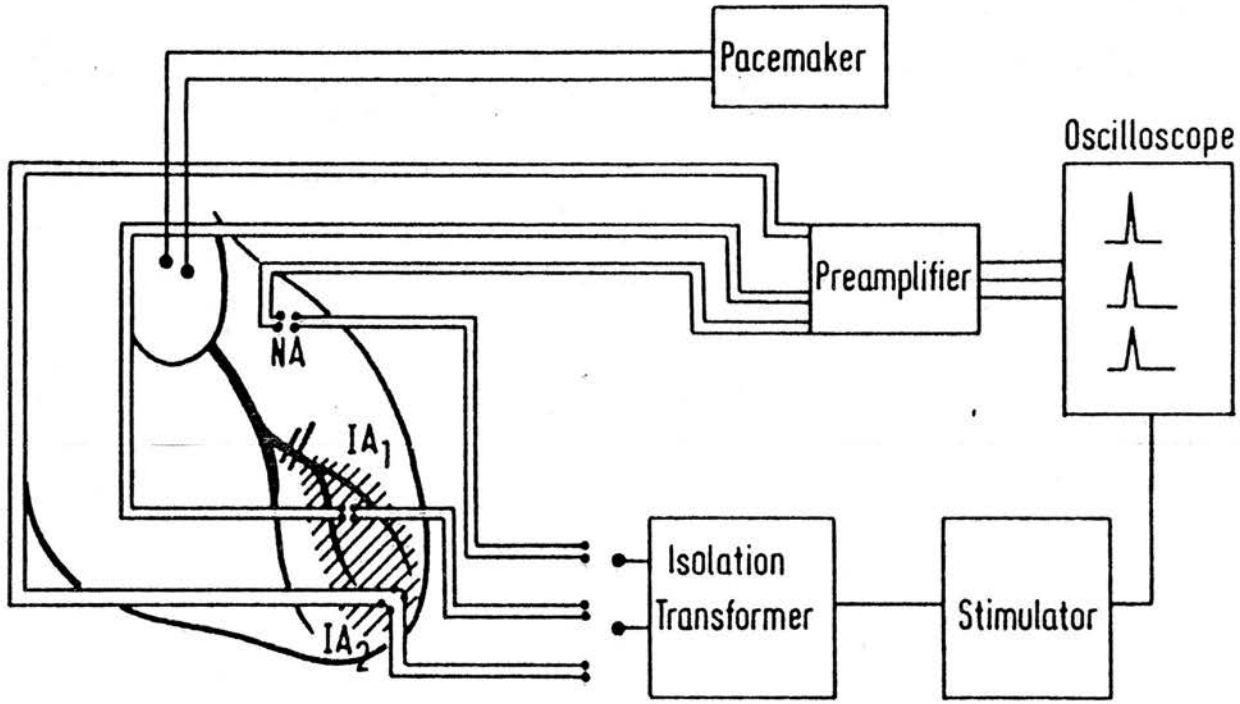


FIG. 11. Experimental arrangement for determination of regional ventricular refractory periods during myocardial ischaemia. Two pairs of bipolar electrodes are attached to epicardium in normal myocardium (NA), in a centrally ischaemic area ($IA_1$) and a border ischaemic area (IA2). One pair of electrodes at each site provides a local bipolar electrogram and the other pair is used for application of extrastimuli

cradle the major diagonal branch of the left anterior descending coronary artery was dissected free at its origin and a loose ligature placed around it. A test occlusion was then performed using a meningeal clip in order to deliniate the visible area of cyanosis on the epicardium. In the absence of any visible change, as occurred in a few dogs due to intense collateralisation, the occlusion was placed more proximally and the procedure repeated. In one or two dogs it was necessary to tie off some large collateral vessels.

Pairs of fine insulated copper hook electrodes (0.13 mm diameter) were inserted in the subepicardium in each of three areas of the left ventricle; a non-ischaemic area (NA), an area approximately at the centre of the potentially ischaemic zone (central ischaemic area or CA), and one at the periphery adjacent to the apex of the left ventricle (peripheral ischaemic area or PA). Two pairs of electrodes were applied at each site, the tips of each electrode being 2 to 3 mm apart. In some later studies pairs of surface electrodes were sutured to the epicardium as less time was necessary for subsidence of current of injury following electrode application. At least 30 minutes was allowed to elapse for current of injury to subside after insertion of subepicardial electrodes.

Bipolar electrograms from each of these three sites were displayed simultaneously on a DM 63 oscilloscope. Amplification was achieved using the AC circuits of a purpose-built AC - DC preamplifier (Mr. J. Austin, Department of Physiology) with a frequency range of 50 Hz to 1 KHz.

Overdrive atrial pacing was performed by means of a stimulation unit, isolation transformer and bipolar left atrial electrode at

rates of 175 or 200 beats/min.

The second pair of electrodes at each site served as stimulation electrodes for local FRP determination by the extrastimulus technique. Extrastimuli of 2 ms duration and 1.5 to 2 times threshold voltage were delivered from a purpose-built stimulator to an isolator transformer (or in later studies, the constant current source) with variable delay triggered from the oscilloscope sweep which in turn was triggered either from the atrial pacing pulse or from the earliest R wave of the local electrogram. Pulses were delivered at varying time intervals after local depolarisation commencing in mid-diastole. This interval was progressively shortened by 5 ms decrements from mid-diastole towards the T wave and by 2 ms decrements on the T wave. The stimulus pulse was monitored on the oscilloscope. Ten to 12 beats recovery period was allowed between each stimulus. Threshold voltage was not altered following commencement of a study.

FRP was defined as the time interval from the peak of the R wave of the bipolar electrogram at each individual site to the earliest stimulus producing a propagated impulse. A series of readings were obtained sequentially from CA, BA and NA respectively. Each series of readings could be performed in about one minute or less and were performed in the same sequence on each occasion. Control measurements were taken over a period of five minutes to ensure reproducibility of results and in general test recordings taken at 2 to 5 minute intervals.

Any episode of ventricular fibrillation was managed by 15 Ws DC defibrillation, but measurements immediately following fibrillation and countershock were discarded.

Data was analysed either directly from the oscilloscope using the storage facility at a sweep speed of 20 $ms.cm^{-1}$ or from polaroid

film or by subsequent play back from tape.

Validation of technique

In view of the possibility that multiple applications of local extrastimuli might cause tissue damage and hence alter physiological readings, studies were peformed with multiple repetitive determinations of FRP at current strengths of 2, 4, 8, 16 and 32 times threshold. Results are shown in Table 2. Thirty-five successive FRP determinations were performed at 30 second to one minute intervals at each stimulation strength in one dog. After each series of readings FRP at two times threshold was again measured (Table 3). Heart rate was maintained constant at 175 beats/min.

Very reproducible data was obtained over multiple testing even at large current strengths. A significant effect on FRP at twice diastolic threshold did transiently follow multiple recordings at 32 times threshold. This was well above any current strength used in FRP determination.

Choice of pulse width

Some studies were performed with variations in stimulus pulse width with the facility for variation from 0.03 to 4 ms of pulse width in the purpose-built stimulator.

Strength-interval curves were constructed at constant heart rate for a variety of pulse widths.

An example of one such study is shown in Fig. 12. Curves are shown for pulse widths of 0.86 ms, 2 ms and 4 ms. Excessively large current strengths were required at shorter pulse widths.

In view of the appearance of the characteristic dip in the strength interval curve using a 2 ms pulse and the degree of overlap

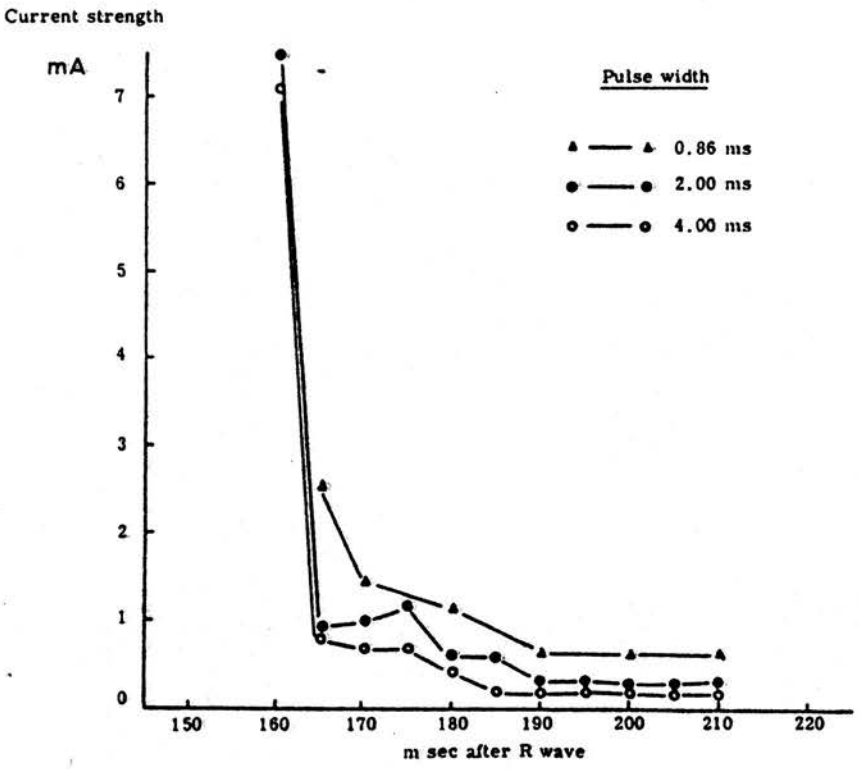


FIG. 12. Strength-interval curves at pulse widths of 0.86 mS, 2.00 mS and 4.00 mS of applied extrastimulus. A characteristic dip in the curve was observed using a pulse width of 2.00 mS

TABLE 2 Effect of successive FRP determinations on FRP at increasing extrastimulus current strengths

| Order of FRP measurement | FRP at diastolic threshold of:- | | | | |
|---|---|---|---|---|---|
| | X2 | X4 | X8 | X16 | X32 |
| 0 - 10 | 143.7 ± 1.3 | 137.0 ± 1.6 | 135.5 ± 0.8 | 128.0 ± 1.0 | 125.7 ± 0.8 |
| 10 - 25 | 143.6 ± 0.9 | 136.8 ± 1.5 | 134.5 ± 0.8 | 126.8 ± 1.0 | 125.4 ± 1.0 |
| 25 - 35 | 143.7 ± 0.5 | 137.2 ± 1.4 | 135.0 ± 0.8 | 126.9 ± 1.1 | 125.4 ± 0.9 |

Mean ± S.D. shown

Values of FRP in mS

TABLE 3 Effect on FRP (determined at an extrastimulus current strength of twice threshold) of 35 previous measurements of increasing current strengths

| Time (min.) | FRP at X2 threshold after 35 readings at threshold of:- | | | | |
|---|---|---|---|---|---|
| | X2 | X4 | X8 | X16 | X32 |
| 0 - 5 | 143.6 ± 0.8 | 143.6 ± 0.9 | 143.0 ± 0.5 | 142.4 ± 0.5 | 136.4 ± 1.8 |
| 5 - 10 | 143.7 ± 0.6 | 143.7 ± 0.9 | 143.0 ± 0.9 | 143.0 ± 0.9 | 141.5 ± 1.6 |

Mean ± S.D. (n = 10)

Values of FRP in mS

*P < 0.01

using a 4 ms pulse, it was decided to perform these studies with pulses of 2 ms duration.

Close reproducibility was observed in studies of successive strength-interval curve determinations at constant heart rate using 2 ms pulse width stimuli.

The time involved in obtaining such a curve (15 - 20 minutes) precluded the use of this technique in studies of acute ischaemia.

Studies therefore were performed of sequential regional FRP determinations over successive 15 minute periods of coronary occlusions and on coronary reperfusion recordings at 32 times threshold. This was well above any current strength used in FRP determination.

Protocols

a) Natural history studies were performed on 15 dogs. Measurements of regional refractory period were made at 2½ minute intervals in normal (NA), central ischaemic (CA) and border ischaemic (PA) areas of myocardium over a control period and during a 15 minute period of coronary occlusion. The LAD coronary artery was occluded at the level of the diagonal branch or in some dogs, just proximal to the bifurcation of the diagonal. Atrial overdrive pacing at 200 beats.min$^{-1}$ was employed. Recordings of FRP were also obtained for 15 minutes after reperfusion.

Episodes of ventricular fibrillation were managed by DC 15 Ws countershock and removal of the coronary occlusive clip. Fibrillation occurred either during occlusion or on reperfusion.

Successive coronary occlusions (up to 3 or 4) were performed in four dogs without intervention, a 30 minute period being allowed between occlusions for recovery.

No biochemical measurements were made in these control studies.

b) The effect of glucose infusion, sufficient to approximately double arterial levels of glucose, was examined in 10 dogs during 15 minute periods of acute ischaemia.

Following a 15 minute sham occlusion two further occlusions were induced with 30 minutes between occlusions to allow for recovery. Saline was infused at a rate of 0.85 $ml.min^{-1}$ over the period of the first occlusion. Ten minutes preceding the second occlusion glucose was infused at 37.5 $mg.Kg^{-1}.min^{-1}$ for 5 minutes and then 17.5 $mg.Kg^{-1}.min^{-1}$ subsequently.

Arterial and local coronary venous blood was sampled at 0, 7½ and 15 minutes of coronary occlusion for determination of glucose, FFA, lactate, sodium, potassium and glycerol concentrations. Insufficient blood samples precluded estimation of all variables at all times.

Regional myocardial blood flow by the radio-actively labelled microsphere technique was determined in five dogs after 7½ minutes of ischaemia during control and glucose treated occlusion.

A third control occlusion with infusion of saline 0.85 $ml.min^{-1}$ was performed in four dogs, a period of at least 45 minutes being allowed to elapse after cessation of glucose infusion to allow normalisation of blood glucose levels.

c) The effect of glucose infusion on FRP determined after 30 minutes of ischaemia was examined in six dogs.

FRP was determined in CA and NA only at 2½ minute intervals before and for 30 minutes after diagonal branch occlusion of the LAD. A bolus injection of glucose (8 G I.V.) was given slowly after 30 minutes ischaemia and FRP determinations continued for 30 minutes.

d) In nine dogs isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ was infused over the period of the second occlusion, the infusion commencing immediately after the control 15 minute post-reperfusion measurement and continued for 30 minutes before occlusion. Arterial and coronary sinus blood samples were taken for analysis of glucose and FFA. Arterial pressure was monitored.

The effect of anti-lipolytic therapy was examined in eight dogs. Following test occlusion (sham) an initial 15 minute control occlusion was performed. Fifteen minutes after reperfusion isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ was infused intravenously and a second coronary occlusion commenced 30 minutes later. Fifteen minutes after reperfusion nicotinic acid 0.5 $mg.Kg^{-1}.min^{-1}$ was infused and isoprenaline infusion continued. Thirty minutes later a further coronary occlusion was made. FRP determinations were made during control, isoprenaline and isoprenaline + nicotinic acid infusions before, during and after coronary occlusion.

Arterial and coronary sinus measurements of glucose and FFA were made.

Critique

The extrastimulus technique for determination of ventricular refractoriness is unsatisfactory in a number of ways. Local inhomogeneity of refractoriness is known to occur between closely adjacent groups of cells during ischaemia (Han, 1969). Refractoriness determined by application of a suprathreshold stimulus over a large number of cells will determine refractoriness of groups of cells showing shorter rather than longer refractoriness in the same zone assuming no exit block. This may not represent average changes in refractoriness in an ischaemic zone. Similarly, the current strength of the applied extrastimulus is of critical importance. Elevation of diastolic excitability threshold may be overcome by elevation of applied current strengths. Prolongation of refractoriness due to post-repolarisation refractoriness, as determined at twice normal diastolic threshold, may be converted to shortening of refractoriness at three times diastolic threshold. A constant current source was used to maintain constant current density at the myocardium-electrode interface and current strength was not altered during the course of a study. Nevertheless, it is possible that some changes in recorded refractoriness relate to variations in

applied test current to the myocardium due to short-circuiting between the electrode terminals. Closely reproducible data was obtained in control studies. The measurement of refractoriness assumes in addition that local depolarisation occurs at the time of the R wave of the local bipolar electrogram. Distortion of the R wave, with in extreme cases, loss of amplitude and fractionation, occurs in ischaemia due to regional fractionation of conduction delay. Recorded shortening of refractoriness during ischaemia may underestimate true refractoriness if delayed depolarisation is not detected. In general the refractory period studies were performed during mild ischaemia often following diagonal branch occlusion of the anterior desceding coronary artery and minimal absolute conduction delays were observed. As with VPB threshold determinations it is assumed that normal recovery of ventricular excitability follows the twelfth interpolated cardiac impulse. Considerably longer periods may be required during ischaemia. The necessity for repeated test stimuli for a single determination of refractoriness precludes rapid sequential measurements. Transient variations, as during the first five minutesof ischaemia, may not be detected. Similarly, simultaneous measurements at the three recording sites is not possible. The same order of electrode testing was used in each study, however, so that at most respective curves of refractoriness will be 30 seconds out of phase with each other.

The choice of area for "central" and "peripheral" zone refractoriness measurement was largely arbitrary based upon the anatomical appearance of the zone of cyanosis following test occlusion. The variability in recordings in these two zones

may relate to some overlap in their electrophysiological properties. The "border zone" of ischaemia in the dog is of the order of 10 mm across (Hearse et al, 1977) and it is unlikely that the "peripheral area" electrodes were placed exactly in the centre of this zone on each occasion. Similarly, the site of "central area" electrodes may not relate to the area of most severe ischaemia, which is, in any case, endocardial and not epicardial.

The difficulties of interpretation of metabolic data have been discussed.

## (c) COMBINED INTRACELLULAR AND EXTRACELLULAR POTENTIAL RECORDING STUDIES

In addition to inhomogeneity of refractoriness, slowing of intramyocardial conduction velocity and the appearance of localised intermittent conduction block should predispose to re-entry and hence be of importance in the pathogenesis of arrhythmias and of VF (Section 2). Extracellular recording techniques have demonstrated striking delays in conduction during acute experimental ischaemia (Scherlag et al, 1974; Cox et al, 1973), but give no information concerning the underlying alterations in transmembrane potential of individual cells which may predispose to such abnormalities. In addition, such recordings cannot demonstrate the appearance of a conduction block at the cellular level.

For these reasons an experimental model has been developed to investigate the association between extracellular and intracellular potential changes during acute myocardial ischaemia, with particular reference to the genesis of arrhythmias and VF.

Considerable difficulty was encountered in the development of the floating micro-electrode technique and thus a description of the developmental studies and validation of the technique is given.

### General description of model

The final experimental arrangement is shown diagramatically in Fig. 13. As in previous studies, the open-chest dog model was utilised and myocardial ischaemia induced by clip occlusion of the left anterior descending coronary artery. It was found more convenient and less traumatic to the vessel in some studies to occlude with a miniature hydraulic-pressure occlusion cuff constructed from fine plastic tubing and connected to a 2 ml syringe.

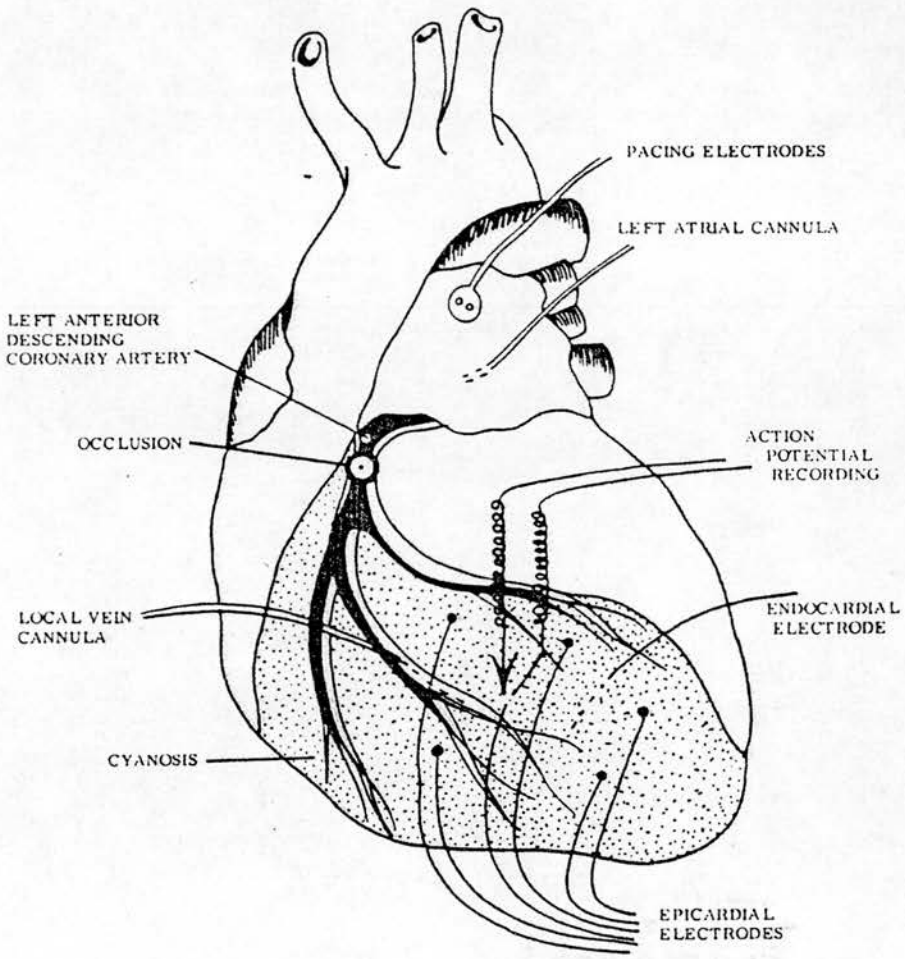


FIG. 13. Experimental arrangement for combined intracellular and extracellular potential recording studies. Atrial pacing electrodes, endocardial and epicardial electrodes and micro-electrodes are shown, together with the sites of introduction of the atrial cannula for microsphere injections and the local venous cannula for blood sampling from the ischaemic zone

A copper wire plunge electrode (0.13 mm diameter), insulated except at the tip, was inserted by means of a needle through the left ventricular wall into the endocardium to record an endocardial electrogram. This electrogram subsequently served to detect the time of endocardial activation.

An independent index of severity of myocardial ischaemic injury is said to be given by the degree of ST-segment elevation in the epicardial electrogram or the summed or averaged ST-segment elevation in several electrograms (Braunwald and Maroko, 1976). Five silver disc electrodes (1.5 mm diameter) therefore were sutured to the epicardium at least 1 cm from the border area of visible cyanosis in the potentially ischaemic zone. (The border zone on occasion may show ST-segment depression). ST-segment analysis was performed on the averaged signals of these five electrograms (vide infra).

Action potential recordings were obtained by the floating micro-electrode technique from the centre of the potentially ischaemic or ischaemic zone. Recordings were used to derive transmembrane potential characteristics. In addition, as the onset of local epicardial activation is given by the time of onset of the recorded action potential, endocardial-epicardial conduction times could be derived by comparison with the endocardial electrogram.

Constant heart rate was maintained in all studies by overdrive atrial pacing to eliminate rate-dependent influences (Surawicz, 1971).

Development of floating micro-electrode technique

The necessity for using glass micro-electrodes for in situ recordings of action potential became apparent after unsuccessful studies with monophasic action potential (MAP) recordings during ischaemia with suction electrodes.

The use of suction electrodes to determine MAP is well described (Schütz, 1931; Hoffman et al, 1959; Olsson, 1971). Several such electrodes were constructed using the cut shaft of a hollow needle as both central terminal and for application of suction with a surrounding ring of fine stainless-steel wire as the second electrode terminal 1 mm distant. Both electrode terminals were embedded in a small dome of dental acrylic.

Studies were performed in three dogs. Suction electrodes were sutured to the potentially ischaemic zone of myocardium. Acceptable and reproducible MAP's were obtained prior to occlusion. Following coronary occlusion, although initial MAP shortening was observed, potentials rapidly developed low amplitude (5 - 10 mV), became indistinct in form and finally disappeared altogether. This should be expected on theoretical grounds as the MAP represents the potential difference between "injured" and surrounding normal tissue. If the latter is "injured" by ischaemia, no meaningful potential may be recorded.

The floating micro-electrode technique has been described (Woodbury and Brady, 1956; Prinzmetal et al, 1962; Czarnecka et al, 1973; Downar et al, 1977) as a means of recording transmembrane potentials from the beating heart, either perfused or in situ. The main problem is maintenance of stable impalements and avoidance of electrode tip breakage in the face of violent myocardial contractions.

The requirement for an experimental model to assess substrate manipulation on the heart is, however, not only for a stable impalement, but also for prolonged and reproducible data over one or more periods of coronary occlusion.

Pilot studies were performed on the beating in situ frog heart using an adaptation of the technique of Woodbury and Brady (1956).

Glass micro-electrodes were prepared following electrode-pulling by boiling under reduced atmospheric pressure in 3 m KCl Standard Jencon's micro-electrode glass tubing (2 mm ext. diameter). Electrodes of resistance 10 - 20 M were selected for use. The distal shaft of the micro-electrode was carefully broken off with forceps and threaded with fine insulated silver wire (0.002"). This, in turn, was soldered to a short piece of coarse silver wire connected to the screened input of a purpose-built high input impedance amplifier (Department of Physiology, Edinburgh University). Potentials were recorded against ground inside a Faraday cage using a DC amplification system. Action potentials were recorded by suspending the distal electrode shaft on the fine silver wire over the surface of the heart, and by means of micro-manipulations gently lowering the micro-electrode onto the epicardium. Gentle tapping often ensured impalement and recording of transmembrane potentials. Full impalements were maintained for only one to two minutes at most, necessitating the use of multiple sequential impalements.

Following studies in the frog, several pilot studies were performed in the open-chest dog preparation by a similar technique. Early experiments were unsatisfactory due to:-

a) Difficulty in impalement of canine epicardium - the weight of the micro-electrode was often insufficient to allow penetration of more than the surface layer of connective tissue cells. Extracellular potentials were easily obtained.

b) Breakage of micro-electrode tip - in the event of impalement of a cell recordings were obtained for, at most, a minute or so due to the relatively violent contraction and movement of the heart within the thorax with respect to the micro-manipulator.

c) Interference - electrical isolation of the dog was attempted by placing the whole animal on an insulated metal plate and within a Faraday cage. It was not possible to ground the whole animal due to biological potentials, e.g. skin potentials, bowel potentials, etc.

d) Extrinsic potential -
the use in these pilot studies of a ground reference resulted in distortion of the upstroke phase of recorded action potentials by extrinsic potentials from excitation of surrounding tissue.

To overcome these practical problems, the experimental design was altered in subsequent pilot studies in the following ways:-

a) To obtain more consistent impalement the electrode shaft was broken more proximally to give greater weight to the electrode. Although achieving this object, it was not possible to support these heavier electrodes with the very fine silver wire. Thicker silver wire (chlorided) was therefore used to suspend the electrode. This in turn added to the rigidity of the system and increased the tip-breakage rate. This was diminished to a certain extent by devising coil or spring or hairpin arrangements with the supporting silver wire. Despite these manoeuvres tip-breakage was common and unacceptable.

b) In order to restrict myocardial movement a thick brass ring (insulated with polythene tubing) was utilised as described by Prinzmetal (1968) and Czarnecka et al, (1973). The ring, c. 10 cm diameter, was sutured to the epicardium and held rigidly in place by clamp-stands clamped tightly to the operating table. By this means tip-breakage was somewhat reduced. It was considered, however, that the distortion of myocardial motion, restriction of contraction within the area of brass ring and probable secondary effects on regional blood flow rendered this technique impracticable. An alternative technique adopted in some studies was that of a tripod sitting on the left ventricular epicardium from which the micro-electrode was suspended. Tip-breakage remained high. Attention was therefore diverted towards the construction of the micro-electrode.

c) Problems of interference and extrinsic potentials were in large part resolved by the use of a second micro-electrode applied closely ( $< 1$ mm) to the first, but without cellular impalement as the reference electrode. The differential transmembrane potential recording, particularly during ischaemia, was then independent of gross changes in DC level.

In order to eliminate the persistent problem of repeated electrode tip breakage a series of studies were performed with different varieties of micro-electrode glass and differing modes of

electrode pulling. Increasing the degree of hardness of the glass and increasing electrode resistance (to 30 - 60 M Ω ) did not improve tip breakage rate.

Better results were achieved with a combination of thicker glass tubing and a two-phase micro-electrode pull. Jencon's 2.5 mm external diameter and 1.5 mm internal diameter was selected. By means of a slow initial electrode pull almost to a tip, followed by a sudden sharp pull, a fine electrode tip was obtained on a shoulder of considerably thicker glass. Electrodes of 10 - 15 M were selected following filling with 3 M KCl and their distal shafts suspended from a chlorided silver wire bent into the shape of a large hairpin. Impalements of up to five minutes were obtained without micro-electrode tip breakage. A second micro-electrode or, in some instances, the distal end of a fine insul ated silver wire, was applied to the epicardium closely adjacent to the micro-electrode for reference. Inputs from recording and reference micro-electrodes were passed into a high input impedance amplifier and DC-coupled preamplifier and displayed on the oscilloscope. Calibration pulses were introduced between ground and one input.

Absolute transmembrane potentials in excess of 100 mV were elicited on initial impalement, but rapidly declined to between 70 and 100 mV. Potentials in the latter range could be maintained for periods in excess of five minutes. The variability in absolute transmembrane potential is accountable for by a combination of:-

a) partial cellular impalement
b) variable electrode tip resistance or capacitance and tip potentials
c) movement artefact
d) tip breakage

In view of the difficulty in being certain of whether a given transmembrane potential represented a true complete impalement, it was considered that quantitative analysis of action potential characteristics be restricted to measurements of duration or time of initiation of the upstroke and qualitative analysis performed on general morphology, plateau shape, upstroke, etc.

Several pilot experiments were performed of action potential recording from ischaemic myocardium following coronary occlusion using this design of micro-electrode. Clear action potentials were recorded at a time when MAP (from an adjacent suction electrode) was unrecordable. Exact correlation has been shown between action potential duration of MAP and that of the true action potential (Hoffman, 1960). Similarly, pilot studies showed no difference in action potential durations recorded from complete or partial impalements. It was considered, therefore, that for the purpose of the experimental model, continuous assessment of action potential characteristics from one cell, albeit with variations in absolute voltage, should give more information than intermittent multiple impalements from many adjacent cells. Nevertheless, experience showed that replacement of electrodes was still necessary in some instances after one or two minutes of recording.

Reproducibility of data recording from multiple impalements

Errors may result in the analysis of action potentials from multiple sequential impalements in the same area of myocardium due to tissue injury by the electrode or simply the natural random physiological variation in characteristics of closely adjacent cells.

A series of control studies were performed therefore of mutliple impalements of various myocardial sites. Three paper discs

TABLE 4 Action potential durations in mS (mean ± S.D.) at 3 left ventricular sites recorded over 50 successive impalements. Measurements made at 25%, 50% and 90% repolarisation

| No. of impalements | SITE A HR 130 | | | SITE B HR 175 | | | SITE C HR 200 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 25% | 50% | 90% | 25% | 50% | 90% | 25% | 50% | 90% |
| 1 - 10 | 109.0 ± 4.8 | 163.7 ± 5.0 | 203.1 ± 2.6 | 85.7 ± 2.2 | 117.2 ± 2.9 | 148.4 ± 3.5 | 82.9 ± 3.8 | 122.7 ± 4.1 | 160.7 ± 2.0 |
| 11 - 20 | 109.2 ± 1.7 | 161.2 ± 1.6 | 205.3 ± 2.3 | 88.4 ± 7.4 | 122.7 ± 2.1 | 152.4 ± 2.9 | 89.2 ± 1.8 | 126.9 ± 3.3 | 161.2 ± 2.7 |
| 21 - 30 | 110.3 ± 1.2 | 161.0 ± 1.7 | 207.4 ± 2.2 | 89.9 ± 2.3 | 124.0 ± 2.9 | 154.8 ± 2.4 | 87.9 ± 1.3 | 125.4 ± 3.6 | 157.2 ± 3.3 |
| 31 - 40 | 109.2 ± 1.3 | 163.5 ± 3.3 | 207.4 ± 2.5 | 93.0 ± 2.6 | 127.9 ± 3.8 | 156.0 ± 6.8 | 84.2 ± 2.1 | 124.8 ± 3.5 | 160.5 ± 1.8 |
| 41 - 50 | 106.8 ± 3.3 | 164.8 ± 3.7 | 209.0 ± 2.0 | 88.8 ± 4.6 | 122.9 ± 3.1 | 156.0 ± 4.0 | 83.0 ± 2.5 | 127.2 ± 2.9 | 161.2 ± 1.1 |

with central 5 mm diameter holes were placed on the left ventricular surface in three positions and 50 impalements performed at each site. Broken tip micro-electrodes were discarded. In view of the possibility of variations being greater at a lower heart rate, differing paced rates were used at each site. Results of these studies are shown in Table 4. Measurements were made at 25%, 50% and 90% repolarisation.

There was no significant difference in readings obtained in the first 10 impalements compared with the 41st to 50th impalement at any site. Subjectively, a slight prolongation in action potential duration appeared to follow tip breakage at some times.

Effect of tip breakage

Breakage of the fine distal tip of the micro-electrode resulted in loss of action potential amplitude, but did not alter repolarisation times, presumably on account of MAP or injury potential registration. Gross tip breakage - with leakage of blood into the electrode and of potassium into surrounding tissue - reduced MAP amplitude, produced triangular-shaped potentials and greatly shortened repolarisation time. After some time extracellular-type potentials were recorded.

Recording system

The combination of endocardial electrogram, epicardial action potential and multiple epicardial extracellular electrogram recording in the final experimental model permitted continuous analysis of:-

a) Action potential duration
b) Action potential morphology
c) Endocardial-epicardial conduction time
d) Averaged epicardial ST-segment elevation
e) Arrhythmia frequency

The endocardial electrogram signal underwent AC amplification with frequency cut-offs of 50 Hz and 1 KHz. The action potential output from the high input impedence amplifier (unity gain or 0.67 gain, frequency response up to 2 KHz) underwent further DC amplification. Capacitance neutralisation was not performed. Signals from the five epicardial electrograms were passed into a purpose-built averaging device consisting of a network of resistors linked centrally. An averaged electrogram of all five signals was obtained with respect to ground and further amplified (AC 0.1 Hz to 200 Hz).

Signals were recorded on tape and analysed on play-back. Action potential duration was determined at 90% repolarisation. Endocardial-epicardial conduction time was taken as the time from onset of the intrinsic deflection of the endocardial electrogram to the time of onset of the intrinsic component of the upstroke of the epicardial action potential.

Averaged ST-segment elevation was determined by passing signals through an ST-segment computer (Luxton et al, 1977) at real speed. Continous outputs of heart rate and of the voltage difference between the PR segment and a selected point on the ST-segment, i.e. ST-segment elevation were obtained on a Devices paper recorder.

Arrhythmia frequency was derived from these same signals using an arrhythmia computer (Neilson, 1974).

## Protocols

Experiments were designed to study a) the natural history of electrophysiological changes during acute ischaemia and the effects of repetitive coronary occlusions, b) the effect of variations in heart rate on ischaemic electrophysiology and c) the effect of substrate manipulation by glucose and antilipolytic therapy (isoprenaline + nicotinic acid).

a) Following several unsuccessful pilot studies, definitive studies of natural history were performed in 13 dogs.

In eight dogs successive five minute occlusions of the LAD coronary artery were performed proximal to two major diagonal branches. On induction of VF the occlusive clip was removed and the heart defibrillated by DC shock (20 W-s).

Up to seven coronary occlusions were performed in each dog with a period of 30 minutes between occlusions to allow for recovery.

Atrial overdrive pacing at a rate of 175 beats.min$^{-1}$ was performed throughout.

Data was recorded on tape and subsequently analysed at 0.5 minute intervals (or continuously in the case of computer ST-segment analysis).

b) The effect of variations in heart rate were examined in five dogs, again with a series of five minute high LAD coronary occlusions (proximal to two major diagonal branches).

The first (sham) occlusion was undertaken at the dog's intrinsic heart rate.

The second occlusion was undertaken at a heart rate of 100 beats.min$^{-1}$, achieved by combined left vagal stimulation and left atrial pacing. Adjustment of vagal stimulation prevented atrioventricular block. During third and subsequent occlusions vagal stimulation was continued at the same strength but the heart rate increased by increments of 20 beats.min$^{-1}$ to a maximum of 200 beats.min$^{-1}$ for the seventh and final occlusion.

c) The effect of glucose was examined in 18 dogs. Following initial five minute sham occlusion successive occlusions of the LAD were performed over five minute periods or until such time as VF occurred. VF was terminated by release of the occlusive clip and immediate DC

countershock (20 Ws). Acid-base balance following countershock was monitored by arterial $PO_2$, $PCO_2$ and pH measurements, but in no case was corrective therapy required. A period of 30 minutes was allowed between occlusions for recovery, but at least 45 minutes after glucose infusion to allow normalisation of blood glucose levels.

Two degrees of coronary occlusion were performed in different groups of dogs.

In group A (11 dogs) the LAD coronary artery was occluded proximal to the two major diagonal branches to produce severe myocardial ischaemia.

In group B (7 dogs) the LAD coronary artery was occluded between the first and second diagonal branches or at the level of the major diagonal branch to produce more moderate ischaemia.

In view of highly reproducible data over second and subsequent occlusions in control studies (see Results) following high coronary occlusion, measurements were made during second and subsequent coronary occlusions.

Glucose infusion was commenced five minutes preceding the third occlusion at a loading dose of 30 $mg.Kg^{-1}.min^{-1}$ for five minutes followed by a maintenance dose of 20 $mg.Kg^{-1}.min^{-1}$ at the time of occlusions. Infusion was discontinued when the occluding clip was released.

Blood sampling from femoral arterial and local coronary venous cannulae was performed prior to and between three and five minutes of ischaemia for biochemical determination of glucose, lactate, FFA, sodium and potassium. Local coronary venous sampling was not possible in group A on account of the grossly reduced blood flow.

Regional myocardial blood flows were determined by injection of radio-actively labelled microspheres after 3½ minutes of ischaemia in

five dogs of the moderately ischaemic group (group B). Measurements were not possible in the severely ischaemic group (group A) due to the high incidence of VF within five minutes of occlusion.

Electrophysiological measurements were made at 30 second intervals both before and following coronary occlusion by replay of data from tape. These included endocardial-epicardial conduction time, cardiac action potential duration (90% repolarisation) in the central ischaemic zone and mean epicardial ST-segment elevation.

d) The effect of antilipolytic treatment was examined in eight dogs. An initial five minute sham occlusion was performed followed by successive additional five minute occlusions, 30 minutes being allowed between occlusions for recovery. A "moderate" ischaemia was induced by occlusion between the two major diagonal branches of the LAD.

Stimulation of lipolysis was achieved by infusion of isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ commencing 30 minutes prior to the second occlusion. Isoprenaline was continued throughout the second occlusion period and discontinued on reperfusion.

Inhibition of lipolysis was then achieved by infusion of nicotinic acid 0.05 $mg.Kg^{-1}.min^{-1}$ followed after five minutes by additional infusion of isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ for 30 minutes preceding and continued during the third occlusion period. Subsequent controls or cross-overs were not possible due to the long plasma half-life of nicotinic acid.

Blood samples were taken from femoral artery and the local coronary vein, draining the ischaemic area before and between three and five minutes of ischaemia for biochemical analysis of FFA, glycerol, glucose, lactate, sodium and potassium. It was not always possible to obtain every estimate, particularly during ischaemia in view of

low blood flows and consequent sampling difficulties.

Haemodynamic monitoring was performed throughout of femoral arterial pressure, left ventricular pressure (Millar catheter tip micromanometer) and its first derivative dp/dt.

Electrophysiological data was analysed from tape at 0.5 minute intervals before and during coronary occlusion.

## Critique

The major problem in action potential recording using floating micro-electrodes was the maintenance of a full intracellular impalement over the 5 minute period of study. In practice it proved impossible to obtain reliable sequential measurements of absolute transmembrane potential without multiple impalements due to movement artefact tip potentials and partial impalements. Rather than obtaining fragmentary data during short occlusions with multiple impalements in different adjacent cells with probable varying electrophysiological properties, data was accepted from a single impalement at one recording site over the period of occlusion. Action potential duration is unaffected by partial impalement (Hoffman, 1960) and represents duration during full impalement. Similarly, the time of onset of depolarisation is unaffected. Qualitative and not quantitative assessments were made of changes in action

potential amplitude and upstroke velocity and absolute transmembrane potentials were determined. An assessment of local diastolic current of injury (TQ segment depression) as an index of diastolic depolarisation was considered but precluded by use of AC-amplification of the epicardial electrograms. It is possible in some instances that partial impalement progressed to recording of an injury potential by tip breakage. During ischaemia action potential amplitudes and upstroke velocities are very similar to those of injury potentials and are almost impossible to distinguish. It can be said, however, that monophasic potentials could not be recorded by the suction electrode technique at the time of recording potentials of "slow response" morphology with the glass micro-electrode. Prior to ischaemia amplitudes and upstroke velocities of potentials were considerably in excess of those obtained from suction electrodes. Lack of absolute transmembrane potential data does not permit a distinction between "slow response" or depressed "fast response" potentials in potentials of "slow response" morphology.

The choice of site of action potential measurement is arbitrary at the "centre" of the ischaemic zone. Subsequent studies (unpublished) have demonstrated that the most severe area of ischaemia is lateral to the anatomic centre of the cyanotic zone, due to a contribution to blood flow in the ischaemic zone from septal anastomoses. Furthermore, no information is given of regional fractionation of conduction delay or action potential duration. Electrotonic interaction between adjacent cells did reveal, however, alternating effects or changes in action potential morphology attributable to regional effects. The emergence of ventricular conduction block between endocardial and epicardial electrodes could arise without necessarily influencing endocardial-epicardial conduction delay if the wavefront of excitation is able to

by-pass a zone of block or delayed conduction. Recorded data may then grossly underestimate the potentiality for formation of a re-entry circuit. Application of extrastimuli was not necessary in this study, hence data is uninfluenced by rate of recovery of excitability.

Division into 'high' and 'low' occlusion groups of animals may be regarded as physiologically arbitrary in view of the large inter-animal variation in collateral anastomatic circulation in the sheep dog. Mean changes in electrophysiological data and incidence of ventricular fibrillation were considerably greater in the 'high' occlusion group however.

The difficulties in interpretation of metabolic data from arterio-venous differences has been discussed.

# 9. ELECTROPHYSIOLOGICAL EFFECTS DURING ACUTE MYOCARDIAL ISCHAEMIA WITHOUT METABOLIC INTERVENTION

## RESULTS

### Normal VPBT curve

The values of VPBT in non-ischaemic myocardium ranged from 12 to 42 mA with a mean of 28.1 ± 3.6 mA in 14 dogs. Values were closely reproducible in an individual animal.

The relationship between values of VPBT following a single coronary occlusion and the frequency of spontaneous ventricular premature beats produced is shown in Fig. 14. The values of VPBT following coronary occlusion fell to a nadir at around five minutes of ischaemia, thereafter rising once more towards pre-occlusion values. The nadir of the VPBT curve is seen to coincide exactly with the time of peak incidence of spontaneous ventricular premature beats. Similarly, on reperfusion, a further dip in the VPBT curve is observed, again coinciding with release ventricular premature beats.

This pattern of change occurred in the presence or absence of spontaneous premature beats, but in the presence of spontaneous VPB changes were greater.

Examples of VPBT change following occlusion in which arrhythmias did not result are shown in the control data of Fig. 25.

In 12 dogs in whom a more moderate degree of ischaemia was induced VPBT fell from a pre-occlusion value of 28.1 ± 3.6 mA to a minimum of 11.0 ± 3.1 mA at five minutes after occlusion ($P < 0.01$), rising to 22.9 ± 5.2 mA at 10 minutes. A fall at one minute of reperfusion to 20.2 ± 3.4 mA was observed, but this was not significant. Data is shown graphically in Fig. 25 (control points) and in Table 8.

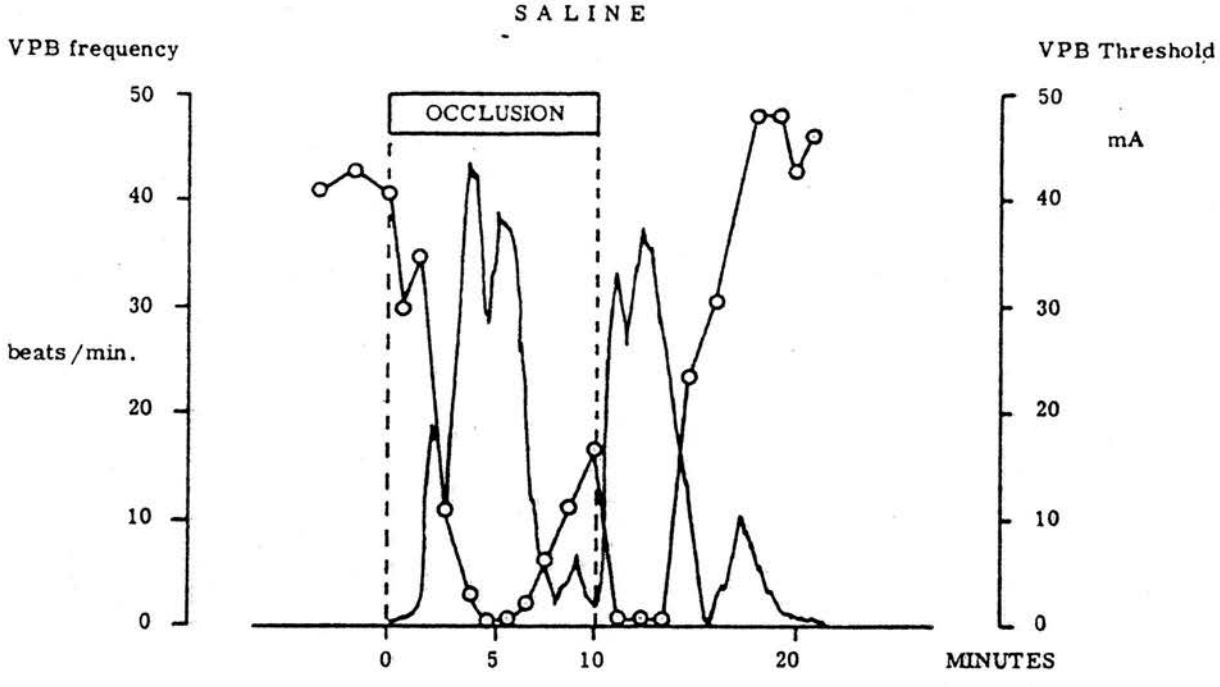


FIG. 14. VPB threshold determinations (open circles) and computer analysis of VPB frequency (continuous line) during and after 10 minutes coronary occlusion. Note the association between the nadirs of the threshold curve and arrhythmias during ischaemia and on reperfusion

Regional refractory periods

Natural history of change in refractoriness during ischaemia

No significant change in FRP was observed during the period of coronary occlusion or after release of coronary occlusion in the normal area (NA) in any dog.

By contrast, a variety of responses was observed in the two ischaemic areas (CA, PA). Representative examples are shown in Fig. 15.

Steady values of FRP were observed prior to occlusion. Following occlusion the following patterns emerge:-

a) A progressive decline in FRP with time (Fig. 15A).

b) A sudden sharp increase of FRP to around 150% of control, followed by a similar progressive decline (Fig. 15B).

c) An initial fall of FRP followed by a rise (Fig. 15C).

d) An initial and sustained rise in FRP (PA, Fig. 15D).

Mean values of FRP in nine dogs are shown in Fig. 15E. The CA shows a mean fall of 10 mS over 12.5 minutes, followed by a rise to near the control value. Patterns (a) and (c) were dominant, the mean values therefore obscuring the opposite directional changes of FRP in the latter half of the occlusion period in different dogs.

More consistent changes occurred in the PA. Mean FRP in PA showed an initial prolongation at 2.5 minutes of 15 mS (N.S.), followed by a progressive shortening (patterns (a) and (b) being dominant).

On release of the occlusion FRP reverted to normal within five minutes. CA, however, showed an overshoot prolongation of FRP, the mean value reaching a maximum of 7.5 minutes ($P < 0.02$). This effect did not attain significant levels in PA.

Gradients of refractoriness

Differences or gradients in FRP were observed between each of the three areas studied as a result of the differing directional changes in FRP in each area, viz. $\Delta$RP CA - PA, $\Delta$RP NA - CA, $\Delta$RP NA - PA.

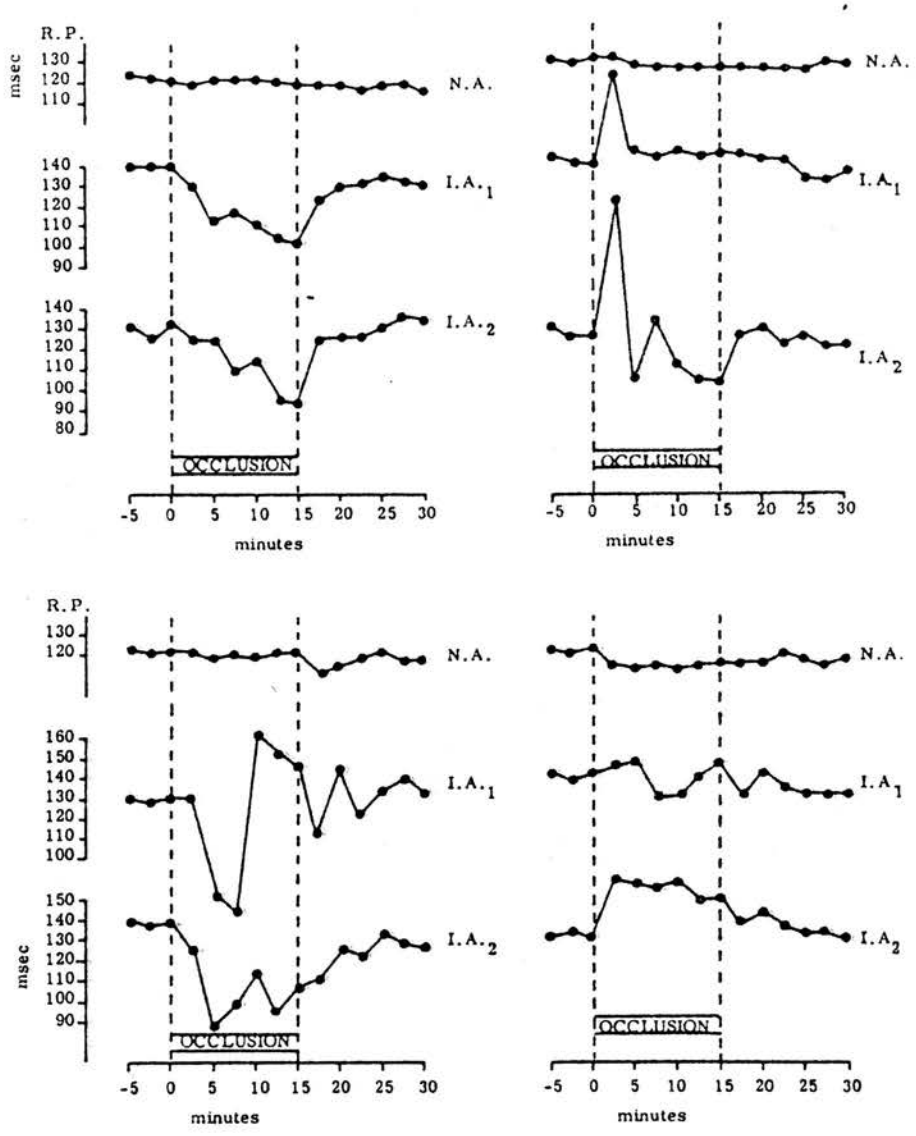


FIG. 15. (a) Patterns of ventricular refractoriness in normal (NA) and central ($1A_1$) and border ($1A_2$) ischaemic myocardium during acute coronary occlusion.
Top left : shortening of RP during occlusion
Top right : sharp initial prolongation of RP followed by shortening during ischaemia
Bottom left : initial shortening followed by prolongation of RP during occlusion
Bottom right : sustained prolongation of RP during occlusion

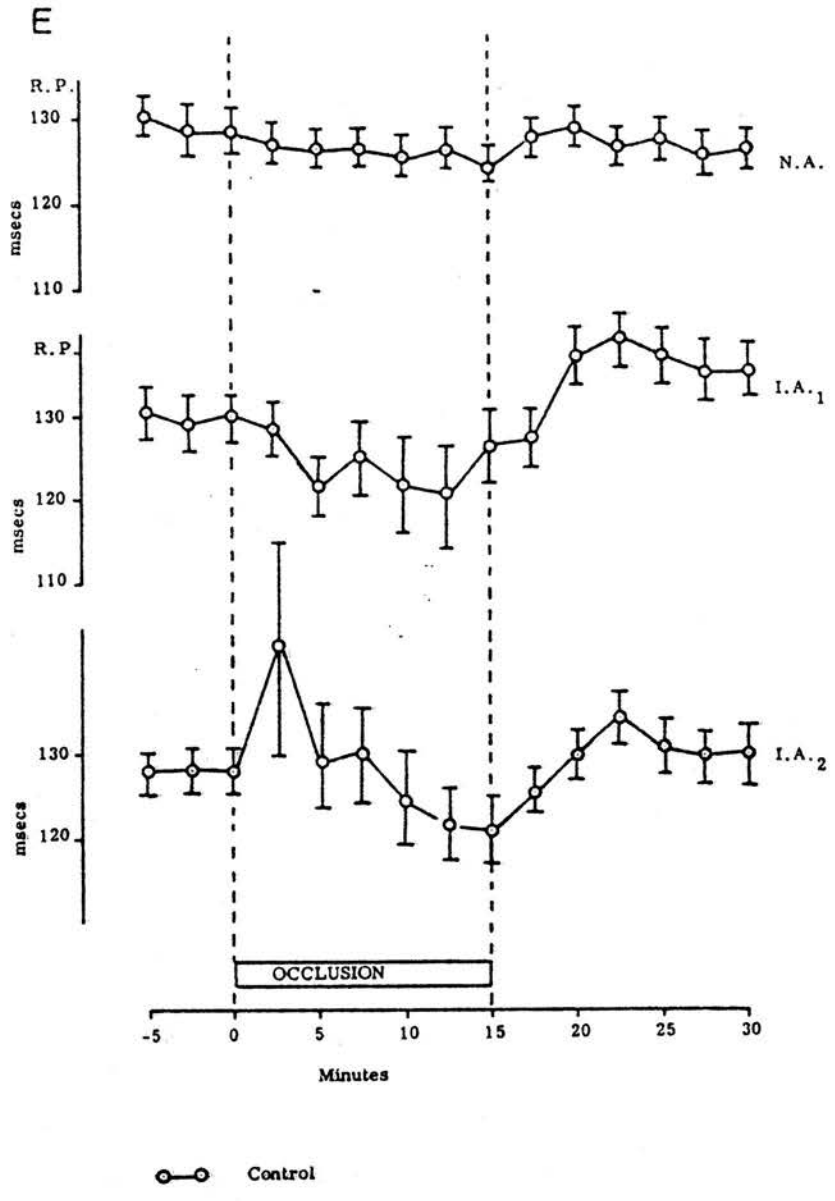


FIG. 15. (b) Mean values of change in ventricular refractory period in normal (NA), central ($1A_1$) and border ($1A_2$) areas of myocardium during acute coronary occlusion (n = 9)

Following coronary occlusion these FRP differences showed a variety of patterns (Fig. 40 control values). Mean values, however, showed an early rise at 2.5 minutes of all three gradients ($P < 0.01$) which reverted to control values by 7.5 minutes. On release a further increase in RP CA - PA was observed, resulting in a small increase (4 mS) in the mean value at 2.5 minutes after release. Spontaneous ectopic beats were noted to occur in some dogs at the time of maximum values of RP gradient.

Ventricular fibrillation

Spontaneous episodes of ventricular fibrillation occurred in six dogs at various times during occlusion or on release of occlusion.

A representative study is shown in Fig. 16. Following coronary occlusion values of FRP in the two ischaemic zones (CA and PA) diverged rapidly. ΔRP CA - PA, the gradient in refractoriness between the ischaemic zones reached a value of 79 mS in this example. In all cases ΔRP CA - PA was at a maximum value as recorded just before VF for spontaneous fibrillation.

An example of changes in FRP preceding reperfusion VF is shown in Fig. 17A. Again just before release the divergence in refractoriness between ischaemic zones was at a maximum.

Mean values of the three RP gradient values for the six dogs grouped about the time preceding onset of VF are shown in Fig. 17C. Values are taken for 2.5 minute recordings preceding VF. A significant rise ($P < 0.02$) in ΔRP is seen during the 2.5 minutes immediately before VF.

On release of occlusion values of ΔRP immediately preceding release were significantly ($P < 0.005$) greater when VF occurred than when it did not. Mean values are shown in Fig. 17B.

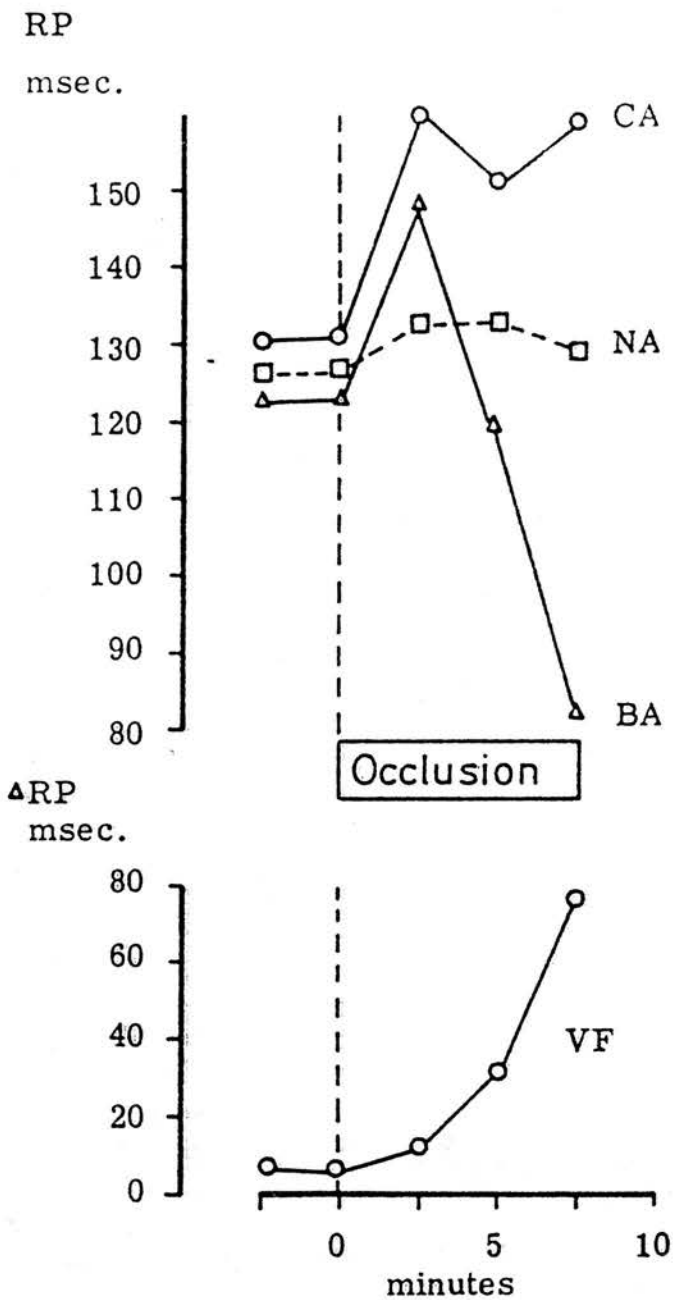


FIG. 16. Example of divergence of refractory period change following coronary occlusion between central ischaemic (CA) and border ischaemic (BA) areas of myocardium preceding ventricular fibrillation in one dog. RP prolongation occurred in CA, whereas shortening occurred in BA. The divergence (ΔRP) between CA and BA of RP is shown in the lower panel and is maximal immediately preceding VF

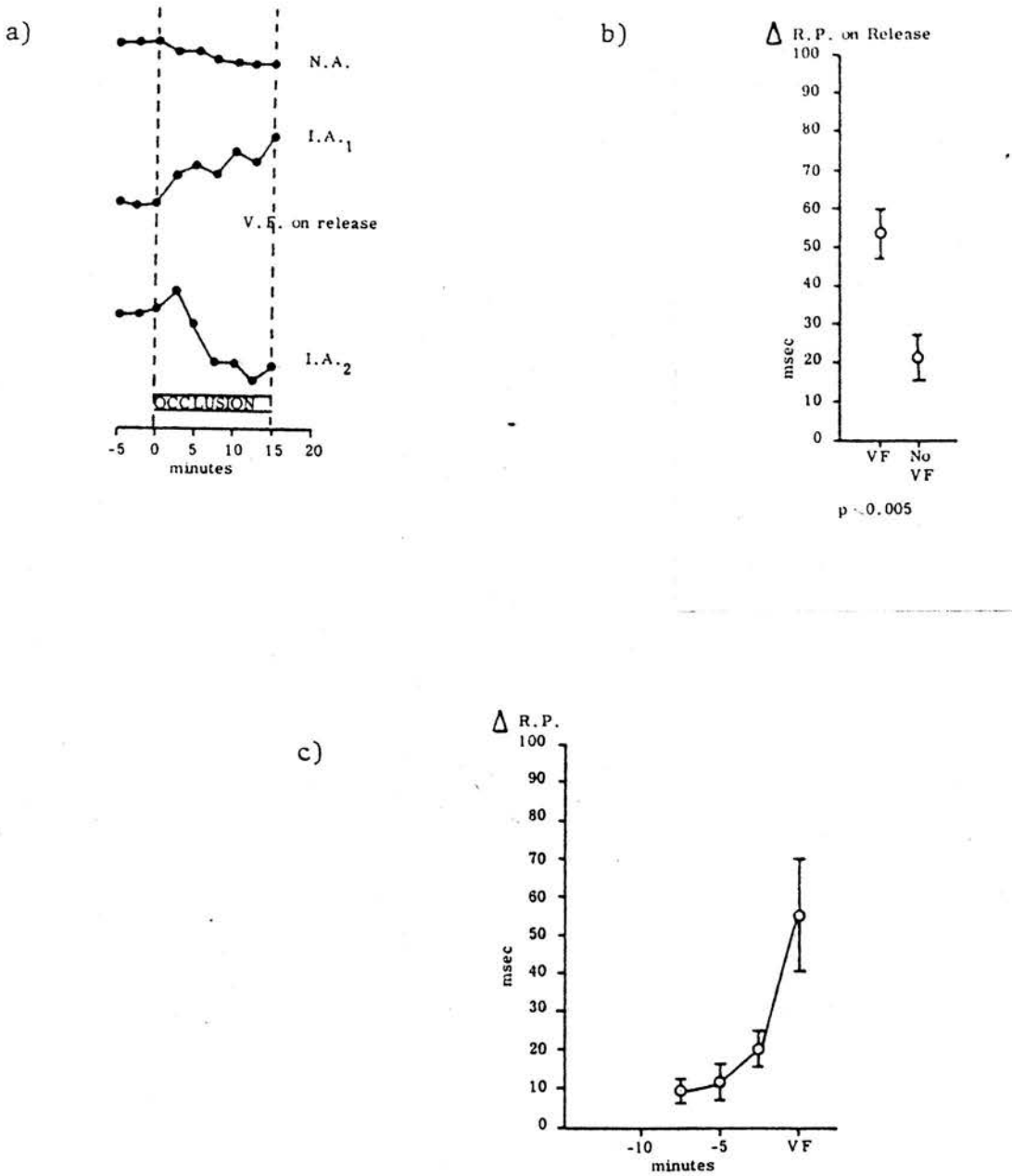


FIG. 17. A. Example of pattern of refractory period change in normal (NA), central ischaemic ($1A_1$) and border ischaemia ($1A_2$) myocardium preceding reperfusion ventricular fibrillation in one dog

B. Mean differences in RP between recording areas immediately preceding release of occlusion, showing significantly higher values preceding reperfusion fibrillation

C. Mean values of gradients of RP ($\Delta RP$) preceding spontaneous fibrillation during coronary occlusion

## Summary

1. The VPBT curve during acute myocardial ischaemia has been defined.

2. Differing patterns of change of ventricular refractoriness occur following acute coronary occlusion in the ischaemic area. Shortening or prolongation of refractoriness or biphasic responses were observed.

3. Prolongation of refractoriness was more prominent in the more severely ischaemic central area, whereas shortening tended to occur in the less ischaemic peripheral area.

4. Differences or gradients in refractoriness occurred between normal and ischaemic areas and within the ischaemic zone. This was greatest at 2½ minutes after occlusion.

5. Refractory periods reverted to normal within five minutes of reperfusion.

6. Spontaneous ventricular fibrillation was directly and significantly related to the degree of dispersion of refractoriness in a given dog immediately preceding fibrillation and following release of occlusion.

## Intracellular and extracellular potential changes

### Natural history

The natural history of electrophysiological change following coronary occlusion varied considerably according to the site of occlusion and severity of resulting ischaemia.

A characteristic sequence of events following proximal coronary artery occlusion is shown in Fig. 18. Each panel shows the epicardial electrogram (mean from five sites), the epicardial action potential, and the endocardial electrogram. Panels are shown at varying time intervals up until onset of VF. The cardiac action potential is recorded from the centre of the ischaemic area. Control potentials are shown in the first panel. The notch preceding the upstroke of the action potential corresponds to the extrinsic potential of Prinzmetal et al (1968). Endocardial activation can be seen to precede epicardial activation. Also no ST-segment elevation is apparent in the control recording.

Following coronary occlusion action potential configuration changes rapidly with shortening, loss of amplitude and disappearance of the initial rapid phase of depolarisation. In panel B of Fig. 18 AP shortening and loss of amplitude is apparent after two minutes of coronary occlusion, together with loss of upstroke velocity. Endocardial-

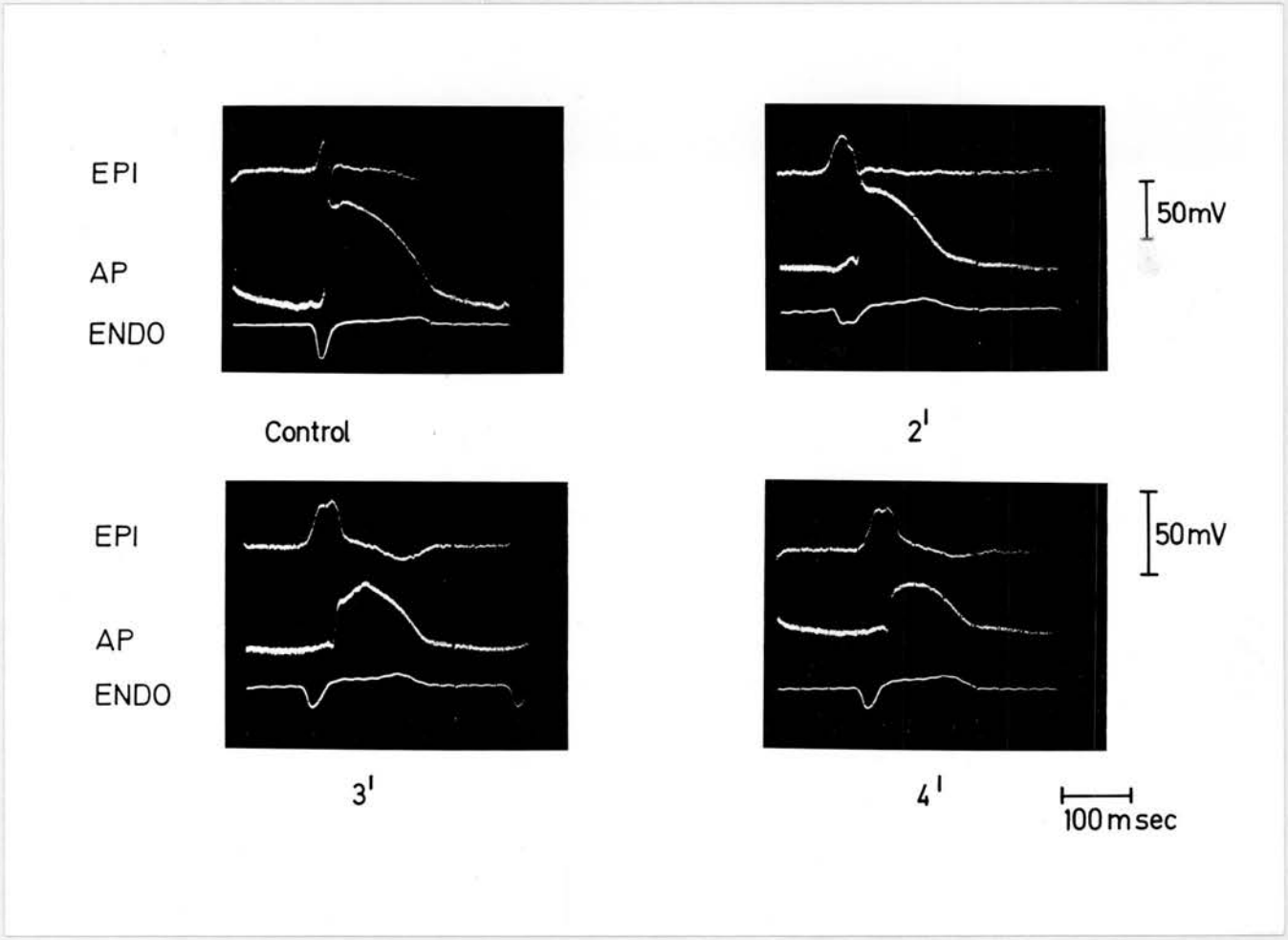


FIG. 18. Recordings of action potential (AP), endocardial (Endo) and averaged epicardial (Epi) electrograms from dog heart in situ before and 2, 3 and 4 minutes after coronary artery occlusion

epicardial conduction time is increased as shown by the delay in onset of the epicardial AP upstroke and widened QRS complex of the epicardial electrogram. By three minutes of ischaemia further activation delay is apparent and potentials assume the morphology of the slow-response type potential described in in vitro studies (Cranefield et al, 1972).

A consistent finding preceding arrhythmogenesis was that of electrical alternans (Fig. 18, Fig. 19). After 3½ minutes of ischaemia alternans of AP amplitude and duration occurred in the example shown, associated with alternans of ST-segment and T wave amplitude in both endocardial and epicardial electrograms. The smaller epicardial AP of a pair was associated with elevation of endocardial ST-segment elevation and increased T wave amplitude, and with decrease in epicardial ST-segment elevation.

In some animals AP and ST-segment alternans proceeded to a pattern of localised 2:1 block or more irregular patterns, this block being recorded only in the intracellular recording (Fig. 18 E, four minute ischaemia). Irregular patterns of block were associated with irregular elevations of the ST-segment and irregularities of T wave morphology in epicardial and endocardial electrograms.

Ventricular premature beats were noted to occur almost invariably at a time of alternating activity with or without recorded block. An example of AP alternation proceeding to VF is shown in Fig. 19.

In no study was any evidence of automatic activity or phase 4 diastolic depolarisation observed during ischaemia.

A quantitative analysis of these changes is shown in Fig. 20. Data are taken from a dog developing VF after four minutes, 30 seconds of ischaemia. Changes in AP duration, endocardial-epicardial conduction

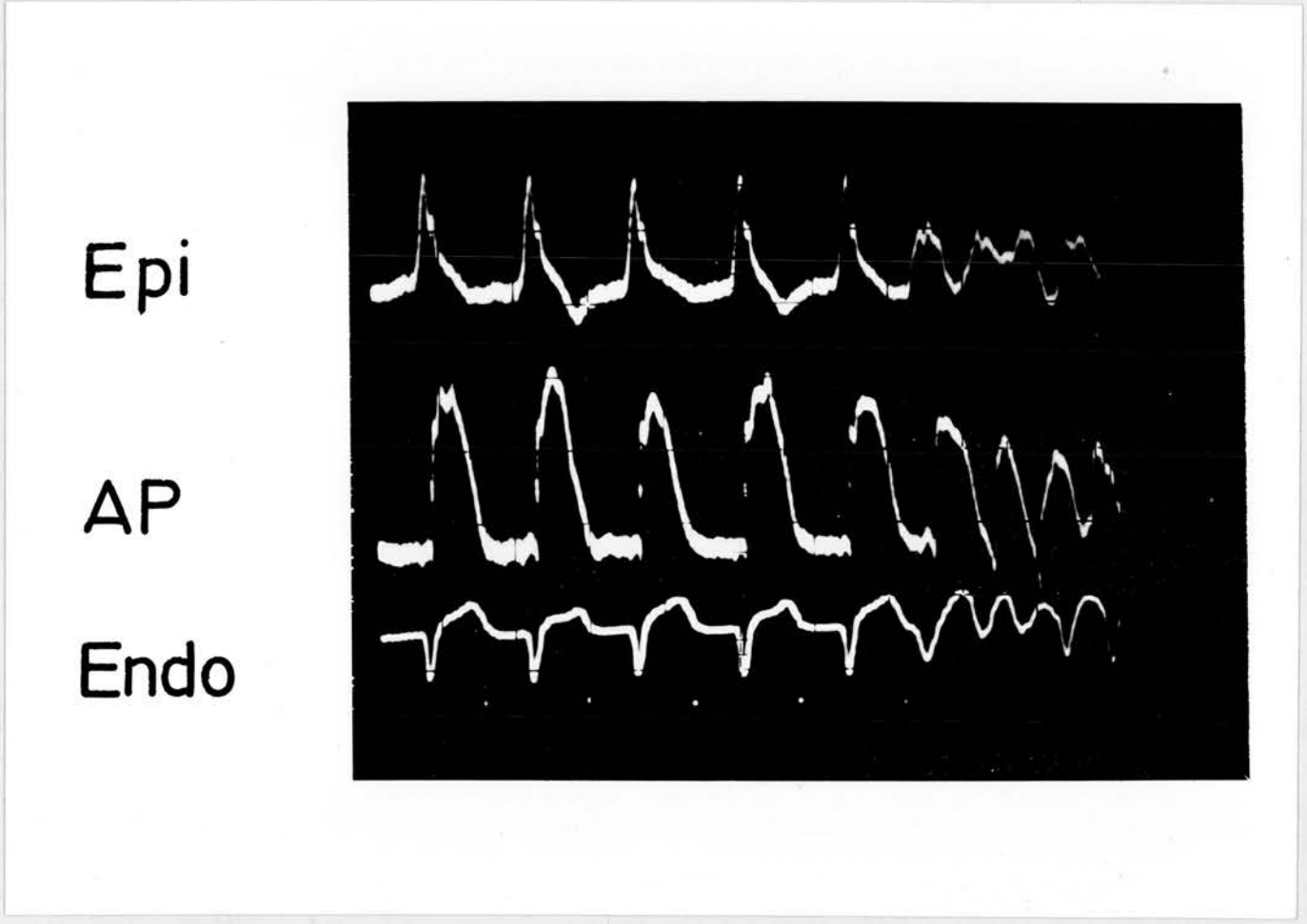


FIG. 19. Alternans of action potential and ST-segment of epicardial and endocardial electrograms preceding ventricular fibrillation

Note the increased asynchrony between intracellular and extracellular recordings at onset of fibrillation

time and mean ST-segment elevation are shown. An initial prolongation of APD (90% repolarisation) was observed after 30 seconds of ischaemia, with thereafter progressive shortening and finally appearance of AP alternans. Endocardial-epicardial conduction time is unaltered for 1½ minutes, but then progressively increased. VF occurs at a time of maximum conduction delay in the ischaemic zone, and maximum AP shortening and alternating activity. 'Slow-response'-type potentials were recorded immediately preceding VF. ST-segement elevation showed progressive elevation until distorted by alternating activity and delayed depolarisation.

Electrophysiological changes following more distal occlusions resulting in "milder" ischaemia produced similar, but less marked directional changes in AP morphology. Patterns of alternation without arrhythmogenesis, AP shortening alone, or even slight AP prolongation alone were observed, depending upon the severity of ischaemia. In mild ischaemia endocardial-epicardial conduction delays were often not observed in the presence of ST-segment elevation and AP shortening. Similarly, ST-segment elevation was observed in the absence of AP shortening.

Initial coronary occlusion

Mean values of AP duration (APD) and endocardial-epicardial CT over the period of an initial five minute occlusion in eight dogs are shown in Fig. 21. Mean values and individual data are tabulated in Table 5. The response to ischaemia was variable despite the consistent application of the occlusion clip proximal to two diagonal branches of the LAD coronary artery. APD shortened from 182 $\pm$ 8 to 140 $\pm$ 15 mS at one minute ($P < 0.001$) and 113 $\pm$ 9 mS at two minutes ($P < 0.001$), although transient prolongation of APD was seen in one dog

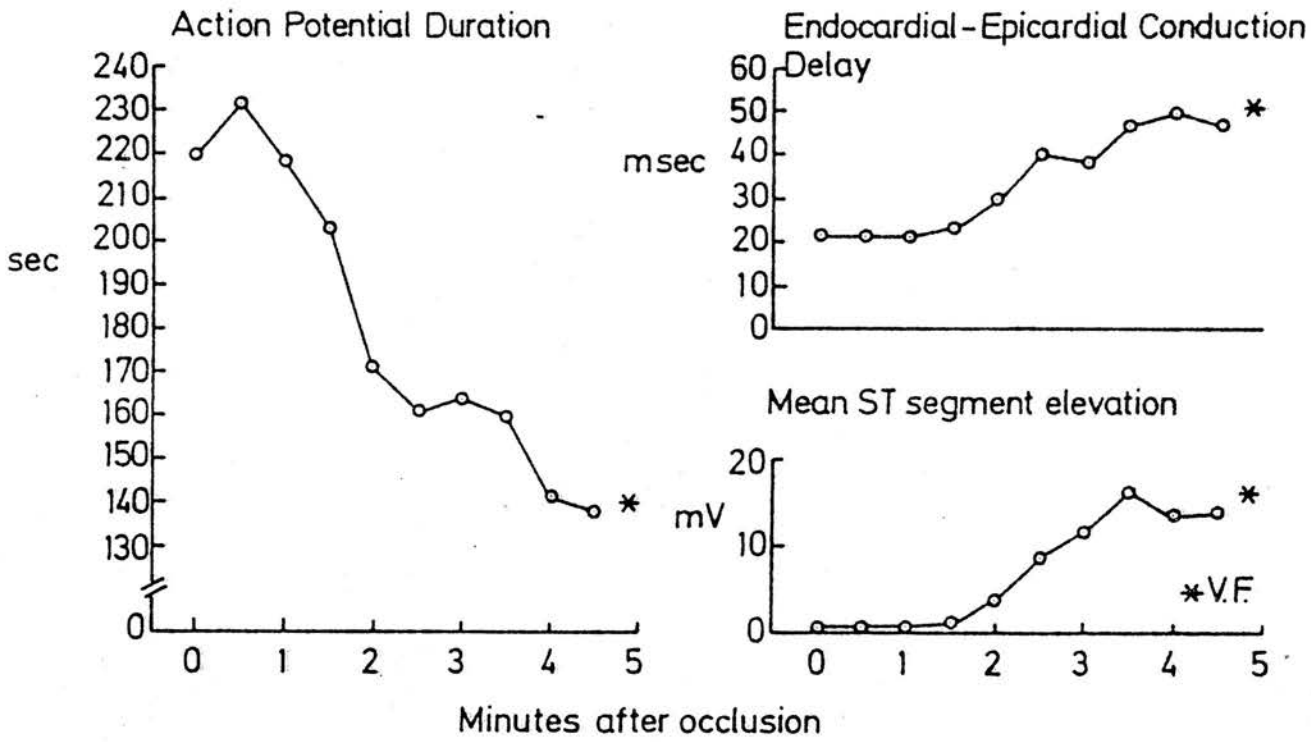


FIG. 20. Changes in action potential duration, endocardial-epicardial conduction delay, and mean ST-segment elevation after coronary artery occlusion. Ventricular fibrillation resulted after 4 min 50 s of ischaemia at a time of maximum recorded conduction delay and action potential shortening

TABLE 5 Effect of initial coronary occlusion on action potential duration and endo-epicardial conduction time

| Dog No. | ACTION POTENTIAL DURATION | | | | | |
|---|---|---|---|---|---|---|
| | Pre-occlusion | Minutes after coronary occlusion | | | | |
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 1 | 220 | 220 | 150 | 140 | 100 | 100 |
| 2 | 150 | 130 | 105 | 150 | 160 | 120 |
| 3 | 190 | 110 | 100 | * | | |
| 4 | 180 | 140 | 150 | 150 | 170 | 155 |
| 5 | 190 | 130 | 80/140† | * | | |
| 6 | 190 | 150 | 120 | 115/160† | 90/160† | * |
| 7 | 155 | 98 | 85/120† | * | | |
| 8 | 185 | 175 | 120 | 120/140† | * | |
| Mean ± SEM | 182 ± 8 | 140 ± 13 | 113 ± 9 | | | |
| | ENDOCARDIAL-EPICARDIAL CONDUCTION TIME | | | | | |
| 1 | 28 | 32 | 48 | 90 | 80 | 25 |
| 2 | 20 | 28 | 42 | 60 | 110 | 80 |
| 3 | 27 | 27 | 62 | * | | |
| 4 | 20 | 20 | 24 | 30 | 32 | 33 |
| 5 | 24 | 44 | 100 | * | | |
| 6 | 20 | 28 | 160 | 165 | 168 | * |
| 7 | 28 | 58 | 75 | * | | |
| 8 | 26 | 28 | 52 | 68 | * | |
| Mean ± SEM | 24 ± 1 | 38 ± 5 | 71 ± 17 | | | |

* = onset of ventricular fibrillation

† = in these cases, alternans of the action potential occurred

within 30 seconds. Loss of action potential amplitude and initial upstroke velocity was observed, but not quantified (see Methods). The appearance of 'slow-response' wave forms occurred earlier in animals where APD shortening was rapid. The progressive shortening of APD was associated in every case with prolongation of CT, mean values being 38 ± 5 mS at one minute and 71 ± 17 mS at two minutes ($P < 0.05$).

Interpretation of epicardial ST-segment change was made difficult by the appearance of widened QRS complexes in five dogs with severe lengthening of CT. However, in three dogs, where lesser degrees of CT were not associated with QRS widening, mean ST increased to 3.5, 5.3, 7.7 and 11 mV at 1, 2, 3 and 4 minutes respectively after coronary occlusion.

VF occurred in five of the eight dogs within the five minute recording period.

Successive coronary occlusions

A comparison of the effect of successive coronary occlusions on APD, CT and the incidence of VF is shown in Table 6 and Fig. 21. After the second coronary artery occlusion, APD shortened and CT lengthened, but at a slower rate than after the first occlusion. Thus APD shortened from 185 ± 9 mS to 157 ± 12 mS at one minute, a length significantly greater than that at one minute after occlusion one ($P < 0.005$). Progressive prolongation of CT was also more gradual than after occlusion one.

There was, however, no significant difference in the rate of change in APD and CT between occlusions 2, 3 and 4. Control APD showed slight successive prolongation prior to successive occlusions despite constant heart rate.

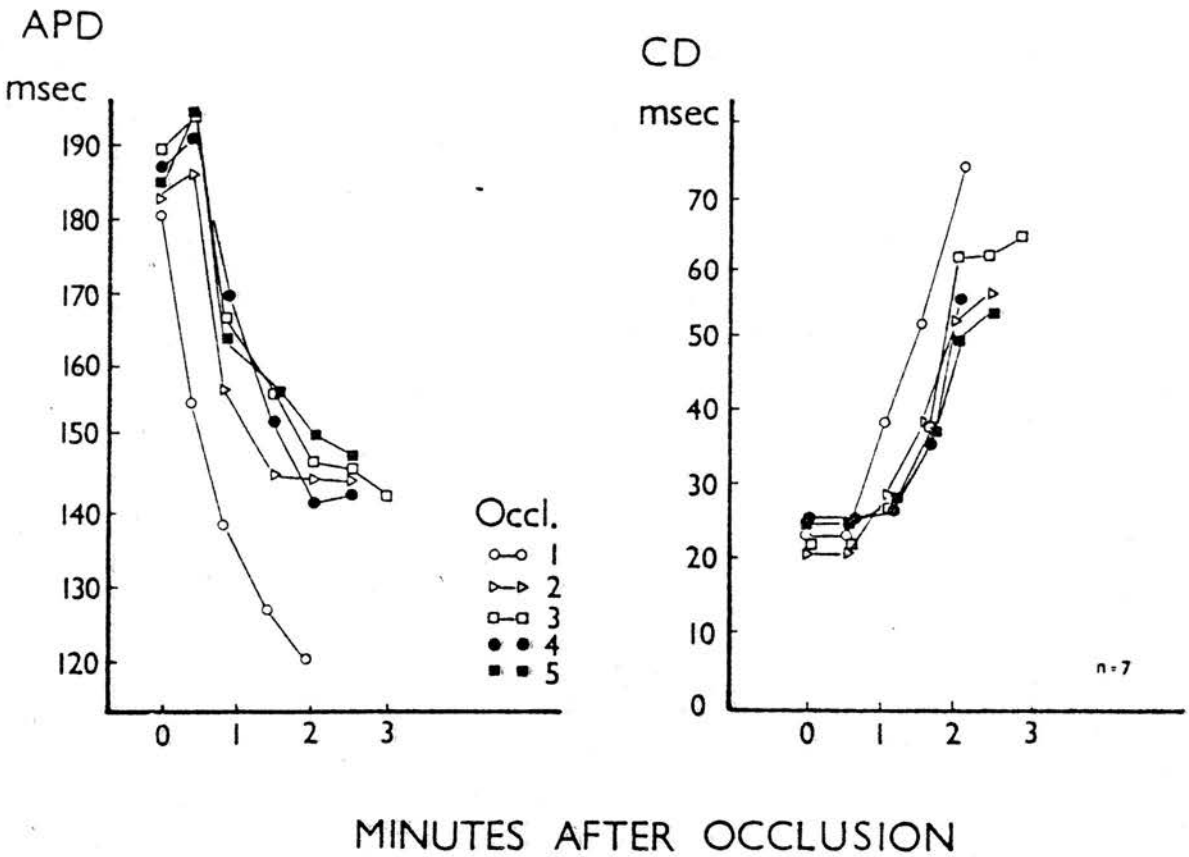


FIG. 21. Effect of successive occlusions on ischaemic changes in action potential duration (APD) and endocardial-epicardial conduction delay (CD). Mean values from 7 dogs are shown.

Changes following the initial coronary occlusion were significantly greater than those during second and subsequent coronary occlusions

TABLE 6 Effect of successive coronary occlusions on action potential duration, endocardial-epicardial conduction time and incidence of ventricular fibrillation

| | Occlusion No. | Pre-occlusion | Minutes after coronary occlusion | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| APD | 1 | 182 ± 8 | 140 ± 13 | 113 ± 9 | | | |
| | 2 | 185 ± 8 | 157 ± 12 | 143 ± 9 | | | |
| | 3 | 191 ± 8 | 170 ± 8 | 147 ± 6 | 136 ± 7 | | |
| | 4 | 190 ± 9 | 169 ± 8 | 140 ± 5 | | | |
| | 5 | 188 ± 12 | 177 ± 13 | 153 ± 9 | | | |
| CT | 1 | 24 ± 1 | 38 ± 5 | 71 ± 17 | | | |
| | 2 | 24 ± 2 | 29 ± 4 | 51 ± 6 | | | |
| | 3 | 25 ± 1 | 27 ± 1 | 49 ± 5 | 68 ± 5 | | |
| | 4 | 25 ± 1 | 28 ± 1 | 51 ± 6 | | | |
| | 5 | 24 ± 2 | 28 ± 3 | 44 ± 9 | | | |
| Number of animals with VF | 1 | - | - | 1 | 3 | 4 | 5 |
| | 2 | - | - | - | 2 | 2 | 3 |
| | 3 | - | - | - | - | 3 | 4 |
| | 4 | - | - | - | 1 | 2 | 4 |
| | 5 | - | - | - | 1 | 3 | 4 |

APD = action potential duration

CT = endocardial-epicardial conduction time

VF = ventricular fibrillation

Mean ± standard error of mean values from 8 dogs are shown

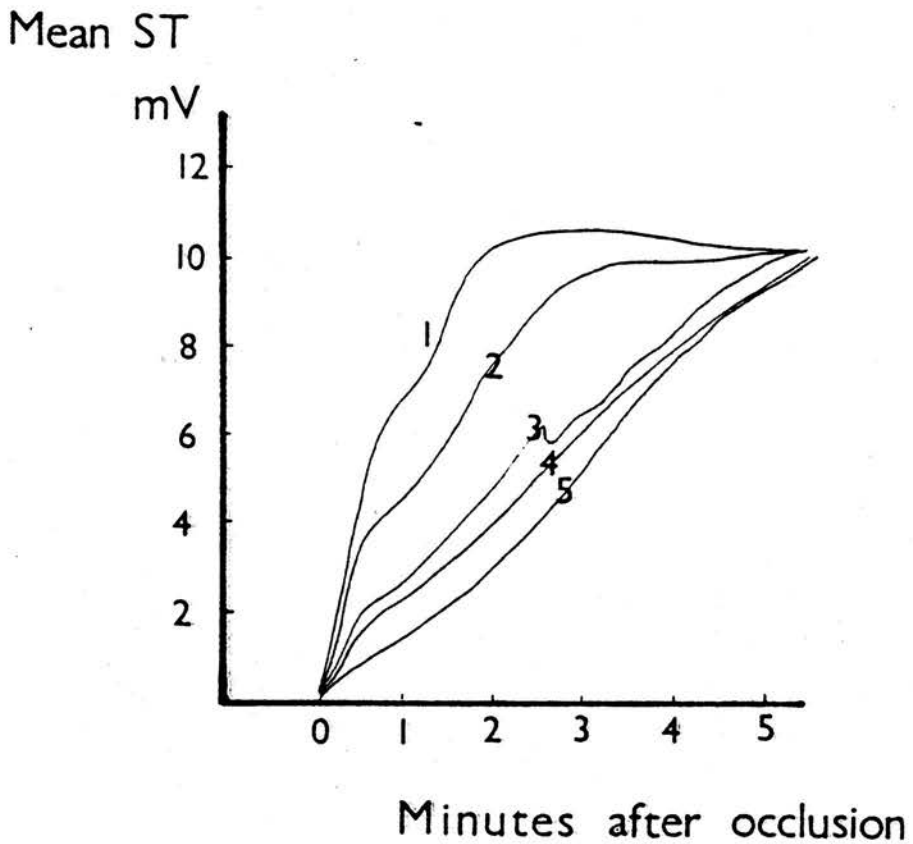


FIG. 22. Effect of successive coronary artery occlusions on mean epicardial ST-segment elevation. More rapid changes in ST-segment elevation followed the initial compared with subsequent coronary occlusions

The slower development of change in APD and CT was also associated with slower development of ST-segment elevation in second and subsequent occlusions in those occlusions not developing gross conduction abnormality. An example of change in mean ST-segment elevation derived from continuous computer analysis of the mean epicardial electrogram is shown in Fig. 22.

Onset of ventricular fibrillation

Ventricular fibrillation followed in five of eight dogs at a mean time of three minutes, 23 seconds after the first occlusion and four minutes, 44 seconds after the second occlusion. It developed in only four dogs after third and fourth occlusions (mean times of onset four minutes, 6 seconds, three minutes, 33 seconds). In each case, this arrhythmia was preceded by the development of action potential alternans - both duration and amplitude. These changes were accompanied by epicardial and by endocardial ST-segment alternans (Fig. 19), and, by varying degrees of conduction blocks with either 2:1, 3:1 or more irregular patterns of endocardial-epicardial conduction block (Fig. 18).

The temporal relations between changes in APD, CT and electrical alternans or conduction block prior to development of ventricular fibrillation are shown in Fig. 23. Data are taken from 20 occlusions in five dogs, all resulting in ventricular fibrillation. Ventricular fibrillation was never seen without shortening of ADP and lengthening of CT. Electrical alternans was seen in 19 instances (95%) and conduction block in 13 (65%).

By contrast during occlusions not resulting in VF, electrical alternans was observed in 72% and conduction block in 33% of

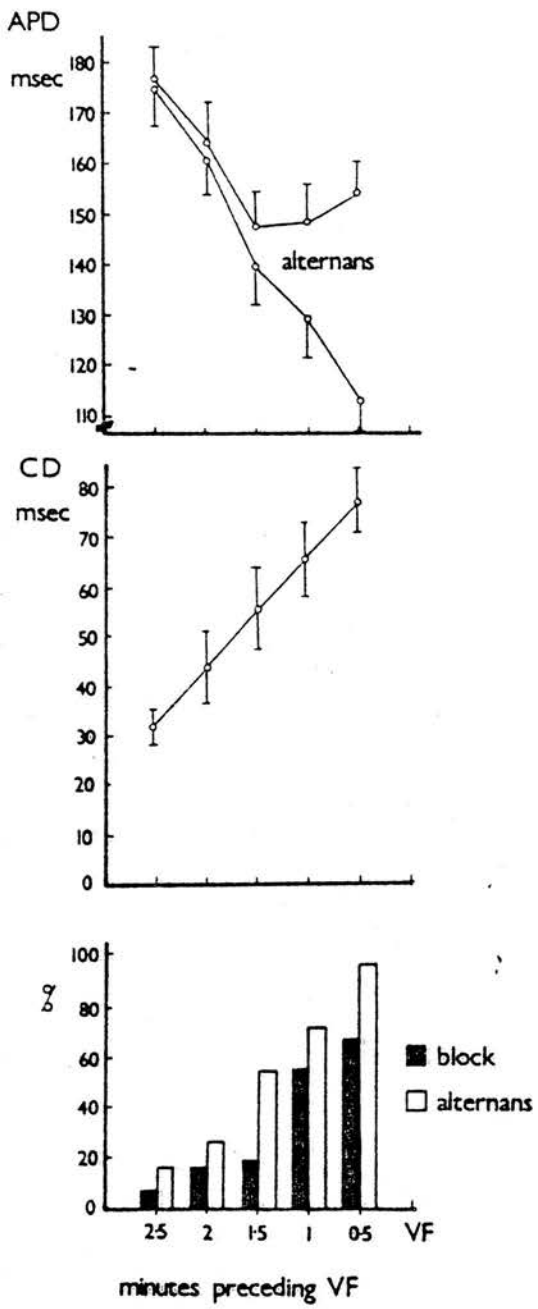


FIG. 23. Changes in action potential duration (APD), endocardial-epicardial conduction (CD) and frequency of occurrence of alternans or block grouped about the time of onset of ventricular fibrillation. Data is taken from 20 occlusions resulting in VF. VF occurred at a time of maximum APD shortening and alternans, maximum CD and maximum incidence of block

instances during the five minute occlusion period. The mean time of onset of alternans was three minutes, 12 seconds and block four minutes, 8 seconds, compared with two minutes, 24 seconds and two minutes, 48 seconds respectively when VF developed.

Effect of heart rate

Mean values of APD and CT during successive coronary arterial occlusions at varying heart rates are shown in Fig. 24 and mean values ± S.E.M. in Table 7.

Controlled heart rates from 100 to 200 beats/minute were achieved with combined atrial pacing and vagal stimulation, during occlusions 2-7 in five dogs. In every case control APD was physiologically dependent on the heart rate, i.e. APD before occlusion was shorter at faster heart rates. After coronary occlusion at slower heart rates, transient lengthening of APD was seen in every case. This was maximal at heart rate 100 beats/min where after 30 seconds APD increased from 224 ± 35 to 245 ± 51 mS. After one minute, APD shortened and CT lengthened, as in the constant heart rate study, but the rate of progression became faster as the heart rate was increased with later occlusions. This was in contrast with the controlled rate study where changes tended to become milder with successive occlusions.

Mean ST-segment elevation similarly showed progressively larger values with increase in heart rate. After two minutes of occlusion mean ST was 1.5, 2.1, 2.6, 6.2 and 6.0 at 100, 120, 140, 160, 180 and 200 beats/minute respectively.

Ventricular fibrillation was not seen at heart rates of 100 to 140 beats/minute, but occurred in one dog at 160 beats/min, two dogs at 180 beats/min and three dogs at 200 beats/min.

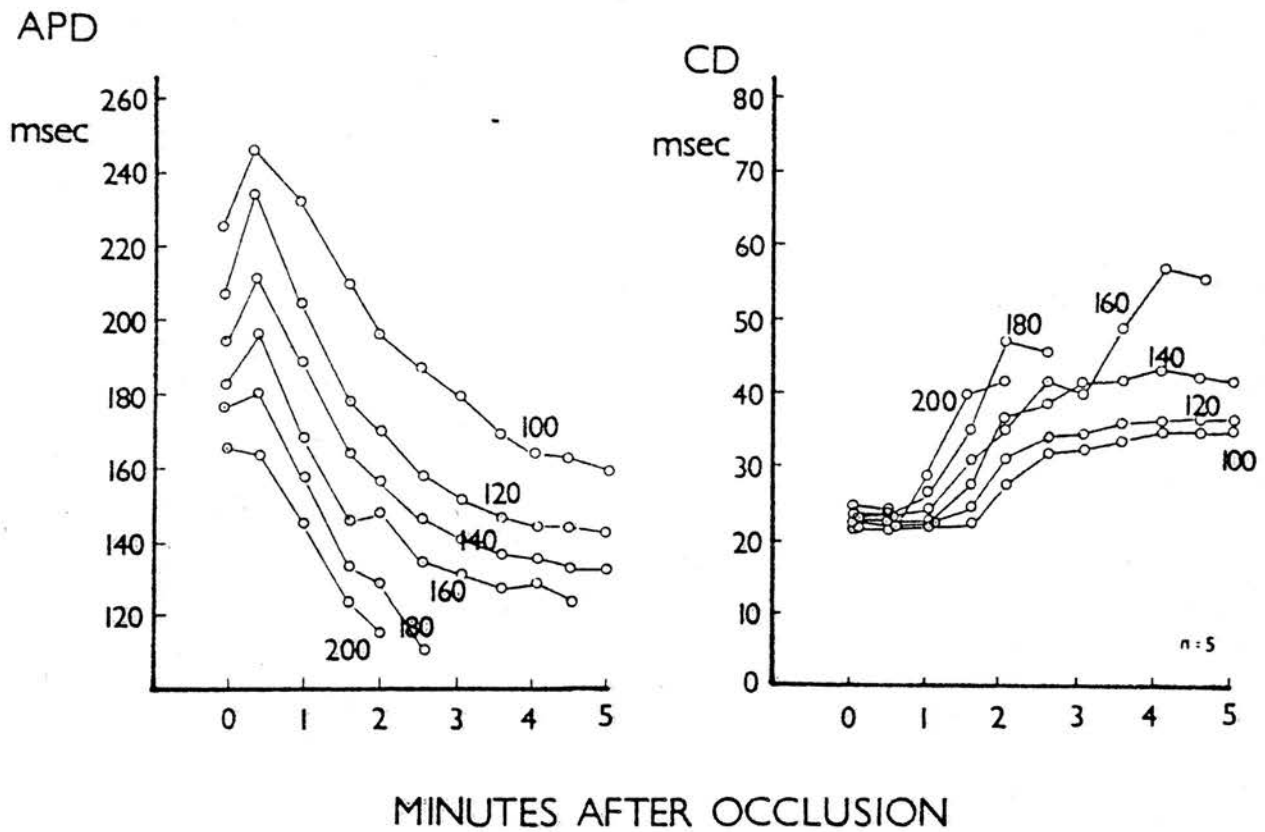


FIG. 24. Effect of varying heart rate on action potential duration (APD) and endocardial-epicardial conduction (CD) following coronary occlusion. Vagal stimulation and overdrive atrial pacing was performed in all cases. Mean values from 5 dogs are shown. Ventricular fibrillation occurred in some dogs at rates of 160 beats $min^{-1}$ and above (see Table 23)

TABLE 7 Effect of heart rate on action potential duration, endocardial-epicardial conduction time and incidence of ventricular fibrillation in 5 dogs

| Heart Rate | Pre-Occlusion | Minutes after occlusion | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| APD mS | | | | | | |
| 100 | 224 ± 15 | 227 ± 22 | 196 ± 27 | 180 ± 25 | 168 ± 27 | 162 ± 26 |
| 120 | 266 ± 14 | 198 ± 20 | 172 ± 21 | 155 ± 19 | 147 ± 19 | 145 ± 18 |
| 140 | 195 ± 12 | 185 ± 14 | 156 ± 15 | 144 ± 14 | 138 ± 15 | 135 ± 15 |
| 160 | 181 ± 7 | 166 ± 7 | 146 ± 12 | 134 ± 17 | 132 ± 11 | - |
| 180 | 175 ± 9 | 158 ± 13 | 126 ± 11 | - | - | - |
| 200 | 164 ± 8 | 147 ± 10 | 119 ± 10 | - | - | - |
| CT mS | | | | | | |
| 100 | 23 ± 1 | 23 ± 1 | 28 ± 2 | 32 ± 2 | 36 ± 3 | 38 ± 3 |
| 120 | 23 ± 1 | 24 ± 1 | 30 ± 3 | 35 ± 3 | 34 ± 4 | 35 ± 4 |
| 140 | 23 ± 1 | 24 ± 1 | 34 ± 2 | 39 ± 3 | 43 ± 3 | 42 ± 3 |
| 160 | 23 ± 1 | 25 ± 1 | 33 ± 2 | 38 ± 6 | 58 ± 8 | - |
| 180 | 22 ± 1 | 26 ± 1 | 46 ± 4 | - | - | - |
| 200 | 22 ± 1 | 27 ± 2 | 42 ± 3 | - | - | - |
| VF | | | | | | |
| 100 | - | - | - | - | - | - |
| 120 | - | - | - | - | - | - |
| 140 | - | - | - | - | - | - |
| 160 | - | - | - | - | - | + |
| 180 | - | - | - | + | + | + |
| 200 | - | - | - | ++ | - | - |

· denotes one episode of VF

Summary

1. Coronary occlusion resulted in transient APD prolongation followed by progressive shortening with loss of AP amplitude and reduced upstroke progressively increasing endocardial-epicardial CT and ST-segment elevation.

2. VF occurred at a time of maximum AP shortening and maximum CT.

3. Significantly greater changes in APD and CT occured during initial, as opposed to second and subsequent five minute occlusions.

4. VF was preceded by electrical alternans in 95% of instances, by intracellularly recorded 2:1 or variable patterns of conduction block in 65% of instances, by progressively increasing endocardial-epicardial CT, and by progressive AP shortening.

5. With increasing heart rate more marked APD shortening, greater CT and an increased incidence of VF followed coronary occlusion.

## 10. ELECTROPHYSIOLOGICAL EFFECTS OF GLUCOSE DURING ACUTE MYOCARDIAL ISCHAEMIA

### Control data - effect of mannitol on VPBT

Mean values of VPBT during infusion of saline and of mannitol during 10 minute periods of ischaemia are shown in Table 9 (12 dogs). To act as control against any possible effect due to successive occlusions, mannitol data is grouped from both group A (mannitol infused during third occlusion) and group B (mannitol infused during second occlusion).

No significant differences in VPBT occurred during mannitol as opposed to saline infusions during ischaemia; that is, no significantly different response of VPBT resulted either as a result of measurements during second or third as opposed to first test occlusion, or as a result of infusing a non-metabolisable sugar.

### Effect of glucose on VPBT

Mean values of VPBT during infusion of glucose are shown contrasted with values of VPBT during infusion of saline in Fig. 25 and Table 8 (14 dogs), and with values of VPBT during infusion of mannitol in Fig. 26 and Table 10 (12 dogs). The comparison between values obtained in groups A and B, in which the order of infusions were reversed, are shown in the inset of Fig. 26 and in Table 11.

In both group A and group B dogs the fall of VPBT after occlusion was delayed and attained higher minimum values during the glucose infusion than during infusion of mannitol. The fall in VPBT in all dogs was significantly reduced after two minutes ($P < 0.05$), after three minutes ($P < 0.01$), after four, five and six minutes ($P < 0.05$) and seven minutes ($P < 0.02$) after occlusion during glucose as opposed to mannitol infusion. There was no significant difference between the two groups after 10 minutes of ischaemia or on reperfusion.

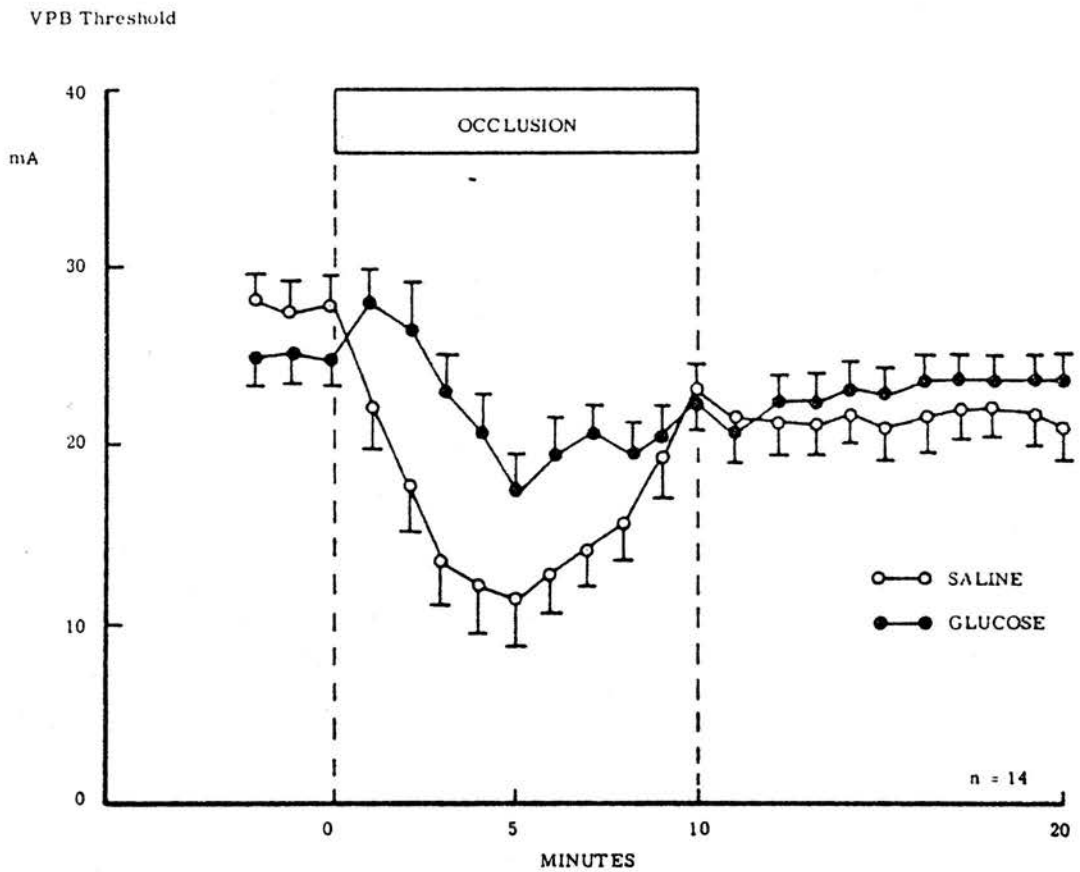


FIG. 25. VPB threshold determinations during 10 minute coronary occlusions during infusion of glucose (closed circles) and saline (open circles)

TABLE 8 Values of VPBT during 10 minute periods of coronary occlusion during saline and glucose infusions in 12 dogs

| Minutes after occlusion | VPBThreshold mA ± S.E.M. | | P |
|---|---|---|---|
| | Saline | Glucose | |
| 0 | 28.1 ± 3.6 | 24.9 ± 2.4 | N.S. |
| 1 | 22.8 ± 5.1 | 28.2 ± 2.1 | N.S. |
| 2 | 17.7 ± 3.2 | 26.4 ± 2.5 | <0.01 |
| 3 | 13.0 ± 2.8 | 22.6 ± 4.7 | <0.02 |
| 4 | 12.4 ± 3.5 | 20.3 ± 5.5 | <0.05 |
| 5 | 11.0 ± 3.1 | 17.2 ± 4.5 | <0.05 |
| 6 | 12.2 ± 2.8 | 19.1 ± 4.9 | N.S. |
| 7 | 13.5 ± 2.7 | 21.2 ± 5.0 | <0.02 |
| 8 | 16.0 ± 2.9 | 19.7 ± 5.1 | N.S. |
| 9 | 19.8 ± 4.0 | 20.1 ± 5.9 | N.S. |
| 10 | 22.9 ± 4.6 | 22.1 ± 6.3 | N.S. |

TABLE 9 Values of VPBT during 10 minute periods of coronary occlusion during saline and mannitol infusion in 10 dogs

| Minutes after occlusion | VPBThreshold mA ± S.E.M. | | P |
|---|---|---|---|
| | Saline | Mannitol | |
| 0 | 31.6 ± 3.0 | 30.6 ± 3.3 | N.S. |
| 1 | 24.8 ± 3.7 | 25.2 ± 4.0 | N.S. |
| 2 | 19.5 ± 3.3 | 20.2 ± 3.5 | N.S. |
| 3 | 14.6 ± 4.1 | 16.7 ± 4.1 | N.S. |
| 4 | 13.9 ± 4.0 | 11.9 ± 3.4 | N.S. |
| 5 | 12.4 ± 3.6 | 10.9 ± 4.1 | N.S. |
| 6 | 12.4 ± 4.0 | 8.5 ± 4.2 | N.S. |
| 7 | 13.4 ± 4.1 | 8.7 ± 4.4 | N.S. |
| 8 | 15.6 ± 4.2 | 10.7 ± 4.4 | N.S. |
| 9 | 18.6 ± 5.3 | 16.9 ± 4.9 | N.S. |
| 10 | 23.5 ± 5.7 | 17.6 ± 4.4 | N.S. |

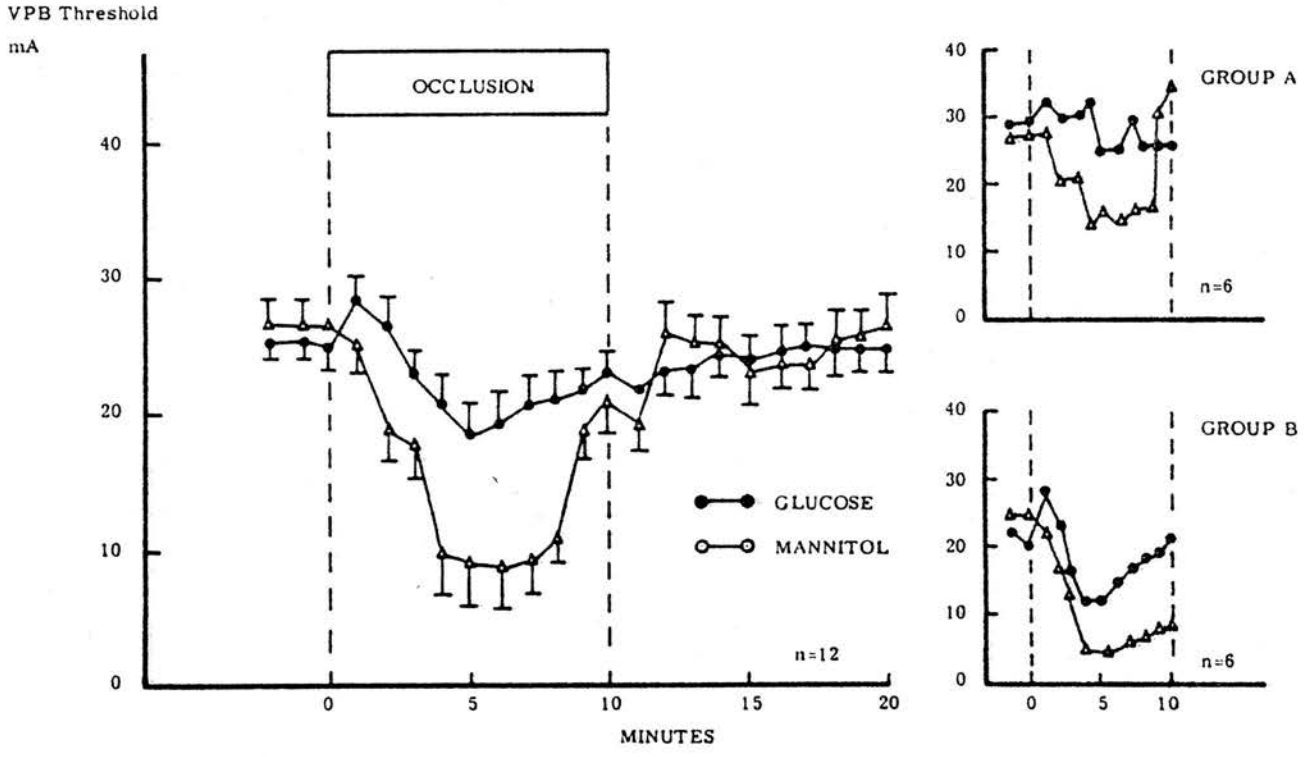


FIG. 26. VPB threshold determinations during 10 minute coronary occlusions in 12 dogs during glucose and mannitol infusions (left) with mean values from group A and group B dogs (right). Threshold was increased during ischaemia by glucose infusion in both groups, but unchanged on reperfusion. Significances were judged by paired t-testing

TABLE 10 Values of VPBT during 10 minute periods of coronary occlusion during glucose and mannitol infusion in 12 dogs

| Minutes after occlusion | VPBThreshold mA ± S.E.M. | | P |
|---|---|---|---|
| | Glucose | Mannitol | |
| 0 | 24.9 ± 2.4 | 27.0 ± 3.2 | N.S. |
| 1 | 28.2 ± 2.1 | 25.05 ± 2.8 | N.S. |
| 2 | 26.4 ± 2.5 | 18.7 ± 2.2 | <0.05 |
| 3 | 22.6 ± 4.7 | 17.2 ± 3.5 | <0.01 |
| 4 | 20.3 ± 5.5 | 8.85 ± 3.2 | <0.05 |
| 5 | 17.2 ± 4.5 | 8.4 ± 4.1 | <0.05 |
| 6 | 19.1 ± 4.9 | 7.4 ± 3.3 | <0.05 |
| 7 | 21.2 ± 5.0 | 8.8 ± 4.5 | <0.02 |
| 8 | 19.7 ± 5.1 | 10.5 ± 3.6 | N.S. |
| 9 | 20.1 ± 5.9 | 19.4 ± 3.8 | N.S. |
| 10 | 22.1 ± 6.3 | 20.1 ± 4.9 | N.S. |

TABLE 11 Values of VPBT during 10 minute periods of coronary occlusion in group A (6 dogs) and group B (6 dogs) during glucose and mannitol infusions

| Minutes after occlusion | VPBThreshold mA ± S.E.M. | |
|---|---|---|
| | Glucose | Mannitol |
| Group A | | |
| 0 | 29.3 ± 2.9 | 29.5 ± 3.4 |
| 1 | 31.3 ± 3.3 | 28.7 ± 3.9 |
| 2 | 30.8 ± 3.7 | 20.5 ± 3.3 |
| 3 | 31.2 ± 4.3 | 20.5 ± 3.3 |
| 4 | 32.0 ± 4.6 | 13.7 ± 4.4 |
| 5 | 25.1 ± 6.9 | 16.0 ± 4.1 |
| 6 | 26.0 ± 7.1 | 13.0 ± 5.3 |
| 7 | 29.3 ± 4.9 | 15.8 ± 6.0 |
| 8 | 25.8 ± 6.8 | 18.7 ± 5.9 |
| 9 | 25.5 ± 6.0 | 34.8 ± 5.9 |
| 10 | 25.3 ± 5.7 | 35.2 ± 5.2 |
| Group B | | |
| 0 | 20.5 ± 2.3 | 24.5 ± 4.5 |
| 1 | 25.2 ± 2.1 | 21.5 ± 5.2 |
| 2 | 22.0 ± 2.5 | 17.5 ± 4.3 |
| 3 | 14.0 ± 4.6 | 14.0 ± 4.6 |
| 4 | 8.6 ± 8.1 | 4.0 ± 1.8 |
| 5 | 9.2 ± 4.5 | 0.8 ± 0.8 |
| 6 | 12.3 ± 4.8 | 1.8 ± 1.2 |
| 7 | 13.2 ± 5.0 | 1.8 ± 1.2 |
| 8 | 13.5 ± 5.2 | 2.5 ± 1.1 |
| 9 | 14.8 ± 5.9 | 4.0 ± 2.5 |
| 10 | 19.0 ± 6.3 | 5.2 ± 2.3 |

Effects on arrhythmias

Spontaneous ventricular premature beats were induced during the first test occlusion in only five out of 14 dogs. In these dogs the mean number of VPB within the first three minutes of occlusion was 14.5 ± 8.5, compared with 0.4 ± 0.39 during infusion of glucose, and 11.4 ± 5.8 during infusion of mannitol. From four to seven minutes of occlusion 37.4 ± 10.9 VPB occurred during saline infusion; 30.2 ± 13.9 VPB followed glucose infusion, compared with 66.6 ± 17.4 after mannitol. Between eight and 10 minutes after occlusion 20.8 ± 10.3 VPB followed saline, 13.0 ± 7.9 VPB followed glucose and 15.2 ± 5.6 VPB followed mannitol infusion.

Effects on osmolality

Arterial plasma osmolality was determined in three dogs and did not differ during glucose (mean 294.2 mosmol) and mannitol (mean 296.1 mosmol) infusion.

Effects on arterial-local-venous differences

The effects of saline, glucose and mannitol infusions on arterial levels and arterial-local-venous differences of glucose, lactate, FFA, sodium and potassium, at the time of VPBT determinations, are shown in Figs. 14 to 18 and in Table 12.

a) Glucose

Arterial levels of glucose were approximately doubled to 13.6 ± 1.08 mM ($P < 0.001$) during infusion of glucose (see Fig. 27), compared with those measured during the control (5.4 ± 0.2 mM) and mannitol (6.0 ± 0.2 mM) infusions.

The arterial-local-venous differences for glucose during control infusions of saline and mannitol were increased at five and 10

minutes after occlusion. During glucose infusion there was a significant increase in the arterial-local-venous difference both before (1.28 ± 0.36 mM, $P < 0.05$) and at five minutes after (3.05 ± 0.84 mM, $P < 0.05$) occlusion compared with controls.

b) Lactate

Arterial and arterial-local-venous levels of lactate are shown in Fig. 28. An excess of local venous over arterial levels of lactate resulted in a negative arterial-local-venous difference for lactate following coronary occlusion. No significant difference was observed between values obtained during glucose or saline infusions at five and 10 minutes of ischaemia. Insufficient data was obtained during infusion of mannitol.

c) FFA

Low arterial FFA levels (53.6 ± 58 $\mu Eq.l^{-1}$) were observed during the first occlusion, i.e. saline infusion (Fig. 29). Infusion of both glucose and mannitol produced a small but not statistically significant reduction in FFA.

No significant differences in arterial-local-venous FFA differences were observed in the three groups.

d) Sodium and Potassium

Arterial and arterial-local-venous differences are shown in Figs. 30 and 31. A significant fall was observed in arterial potassium from 3.6 ± 0.2 mM during saline infusion to 3.0 ± 0.1 mM during infusion of glucose ($P < 0.05$). No significant changes were observed in arterial or arterial-local-venous differences of sodium.

TABLE 12 Metabolic effects following coronary occlusion during infusions of saline, glucose and mannitol

| | MINUTES AFTER OCCLUSION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Saline | | | Glucose | | | Mannitol | | |
| | 0 | 5 | 10 | 0 | 5 | 10 | 0 | 5 | 10 |
| Glucose mM | | | | | | | | | |
| Arterial | 5.43 ±0.19 | 5.50 ±0.14 | 5.62 ±0.18 | 13.59*** ±1.08 | 14.18*** ±1.03 | 14.41*** ±1.05 | 5.96 ±0.21 | 5.28 ±0.22 | 5.17 ±0.28 |
| Local Venous | 4.96 ±0.21 | 4.41 ±0.27 | 4.51 ±0.25 | 12.15*** ±0.96 | 12.39*** ±1.10 | 12.03*** ±0.91 | 5.93 ±0.41 | 4.49 ±0.52 | 4.53 ±0.32 |
| A-lv | 0.58 ±0.17 | 1.17 ±0.28 | 1.45 ±0.36 | 1.28* ±0.36 | 3.05* ±0.84 | 1.87* ±0.58 | 0.51 ±0.26 | 1.27 ±0.35 | 1.30 ±0.22 |
| Potassium mM | | | | | | | | | |
| Arterial | 3.63 ±0.15 | 3.65 ±0.16 | 3.71 ±0.16 | 3.01* ±0.13 | 3.06* ±0.15 | 3.11* ±0.16 | 3.42 ±0.19 | 3.70 ±0.16 | 3.66 ±0.32 |
| Local Venous | 3.82 ±0.15 | 4.27 ±0.26 | 4.30 ±0.28 | 3.29* ±0.07 | 3.85 ±0.33 | 3.68 ±0.19 | 3.35 ±0.14 | 4.35 ±0.28 | 4.07 ±0.33 |
| A-lv | 0.09 ±0.11 | 0.56 ±0.28 | 0.64 ±0.26 | 0.16 ±0.05 | 0.57 ±0.38 | 0.34 ±0.32 | 0.05 ±0.12 | 0.83 ±0.42 | 0.49 ±0.33 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Lactate nM | | | | | | | | | |
| Arterial | 0.81 ±0.22 | 0.77 ±0.21 | 0.78 ±0.23 | 0.50 ±0.12 | 0.74 ±0.25 | 0.75 ±0.28 | | | |
| Local Venous | 0.70 ±0.20 | 1.77 ±0.61 | 1.26 ±0.58 | 0.50 ±0.09 | 1.96 ±0.63 | 1.23 ±0.35 | Insufficient data | | |
| A-1v | 0.18 ±0.16 | -1.02 ±0.55 | -0.78 ±0.42 | 0.07 ±0.14 | -1.08 ±0.61 | -0.28 ±0.41 | | | |
| Free Fatty Acids µEq/1 | | | | | | | | | |
| Arterial | 536 ±58 | 360 ±62 | 452 ±49 | 378 ±39 | 310 ±31 | 358 ±62 | 306 ±38 | 324 ±51 | 278 ±51 |
| Local Venous | 427 ±52 | 347 ±41 | 389 ±88 | 328 ±52 | 301 ±77 | 289 ±72 | 234 ±66 | 176 ±36 | 238 ±52 |
| A-1v | 63 ±92 | 12 ±32 | 69 ±102 | 33 ±65 | 55 ±53 | 12 ±48 | 13 ±28 | 106 ±28 | 45 ±82 |

* $P < 0.05$

*** $P < 0.001$

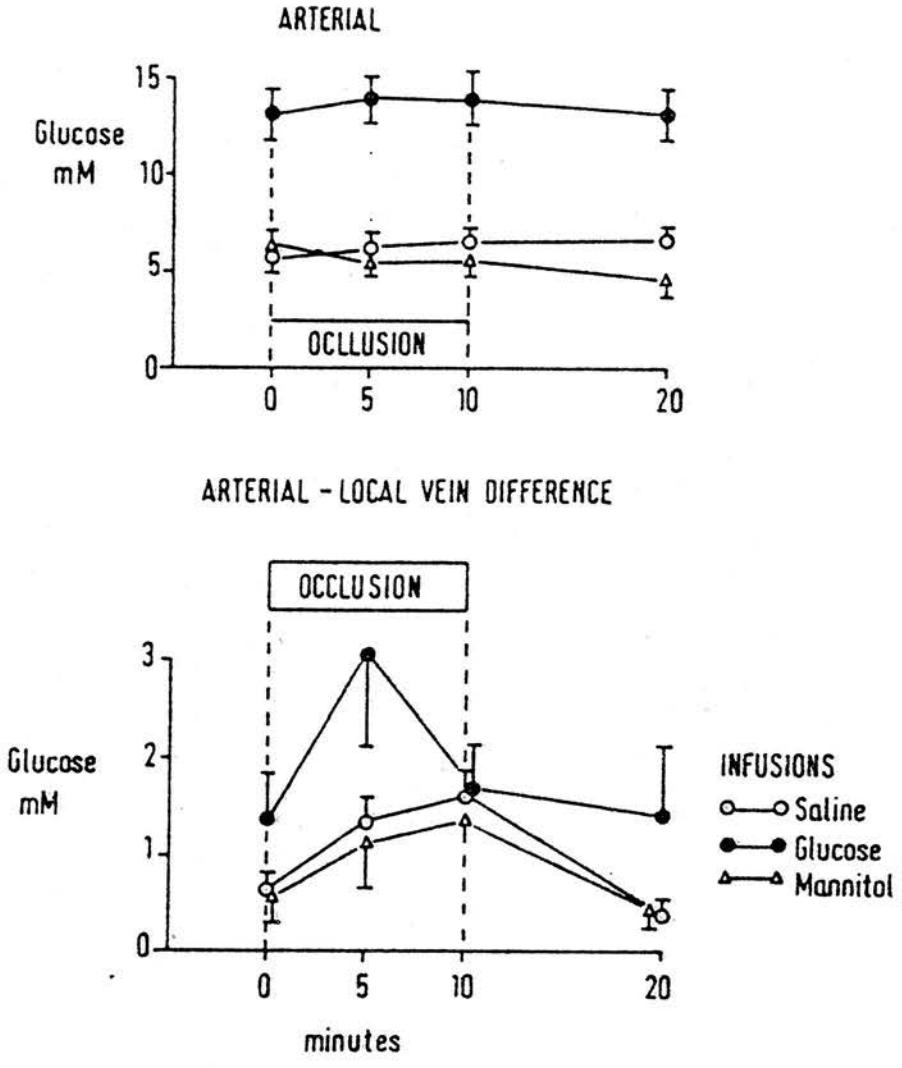


FIG. 27. Arterial and arterial-local venous differences of glucose following coronary occlusion during infusions of saline, glucose and mannitol. Note the increased A-lv difference following occlusion during control infusions of saline and mannitol. Glucose infusion resulted in a further significant increase in A-lv difference at 0 and 5 minutes

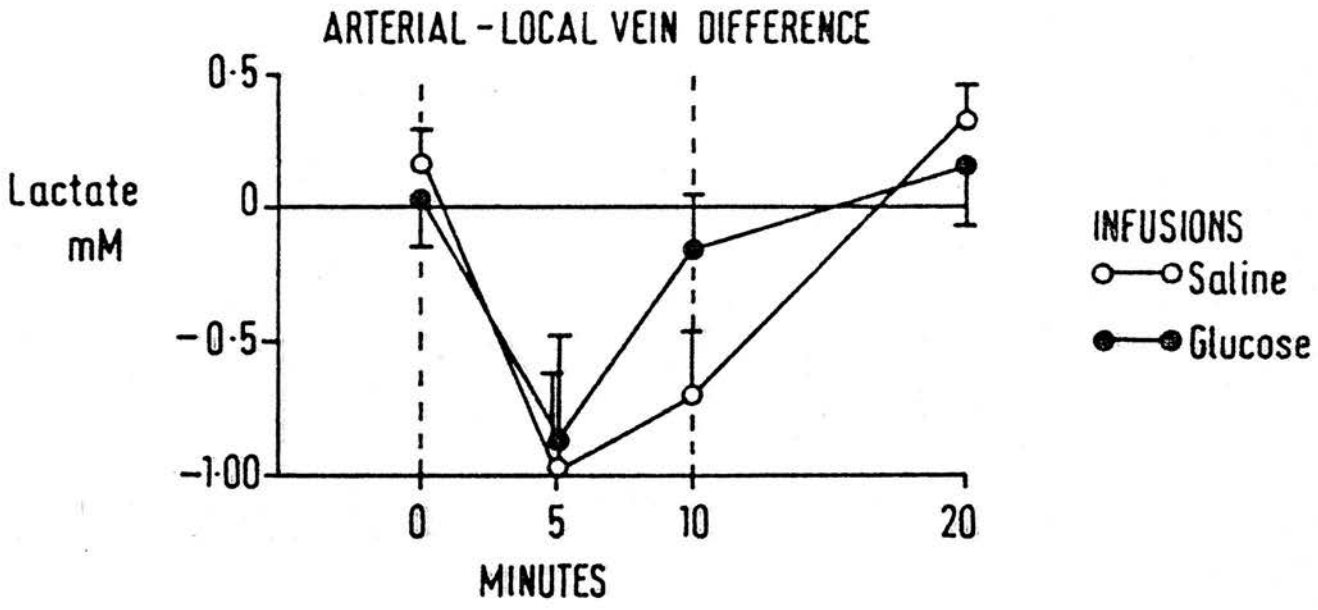


FIG. 28. Effect of glucose infusion on arterial-local venous differences of lactate following coronary arterial occlusion

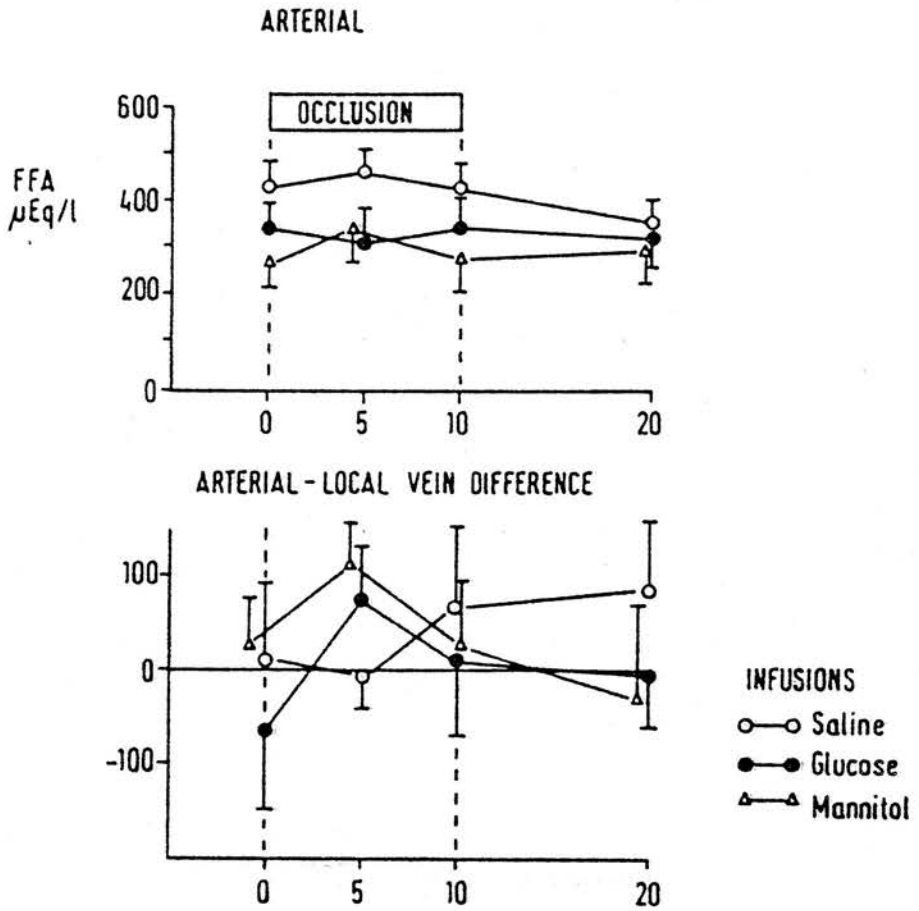


FIG. 29. Effect of saline, glucose and mannitol infusions on arterial and arterial-local venous differences of free fatty acid following coronary occlusion. Arterial levels were low and no significant differences in A-lv gradients observed

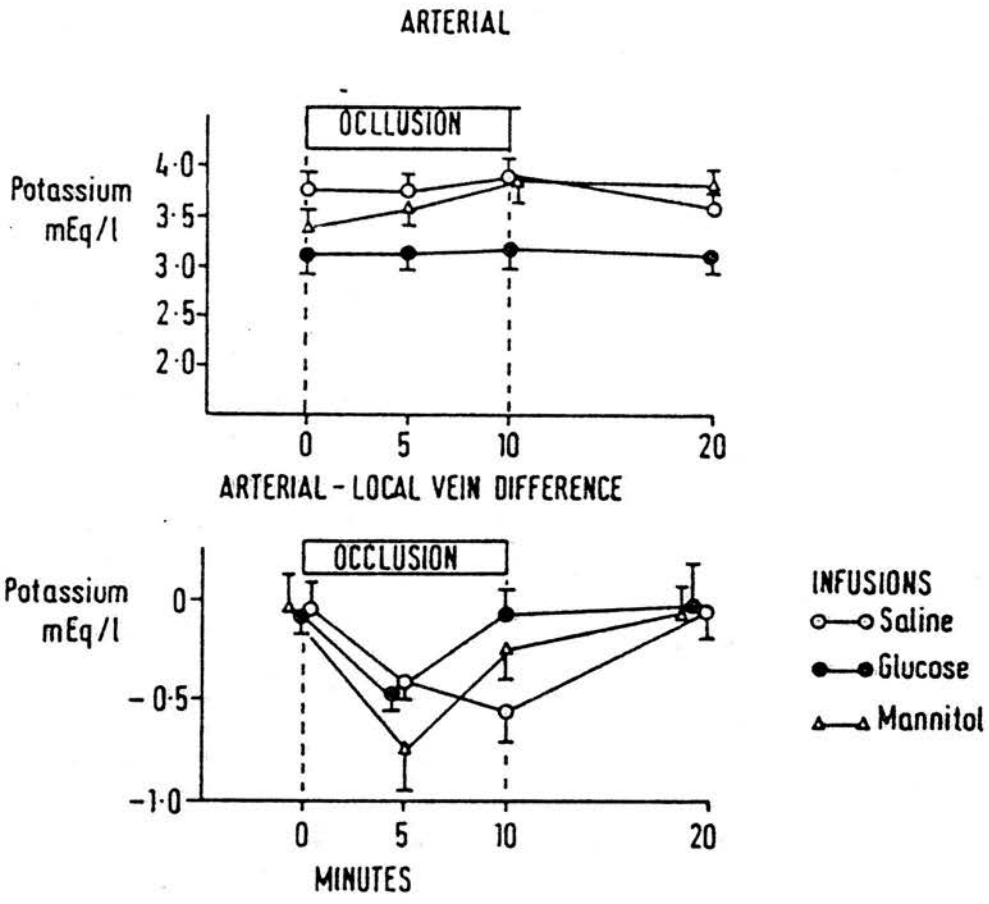


FIG. 30. Effect of saline, glucose and mannitol infusions on arterial and arterial-local venous differences of potassium following coronary occlusion. Glucose infusion resulted in a small, but significant, fall in arterial potassium concentrations, but did not influence recorded A-lv differences

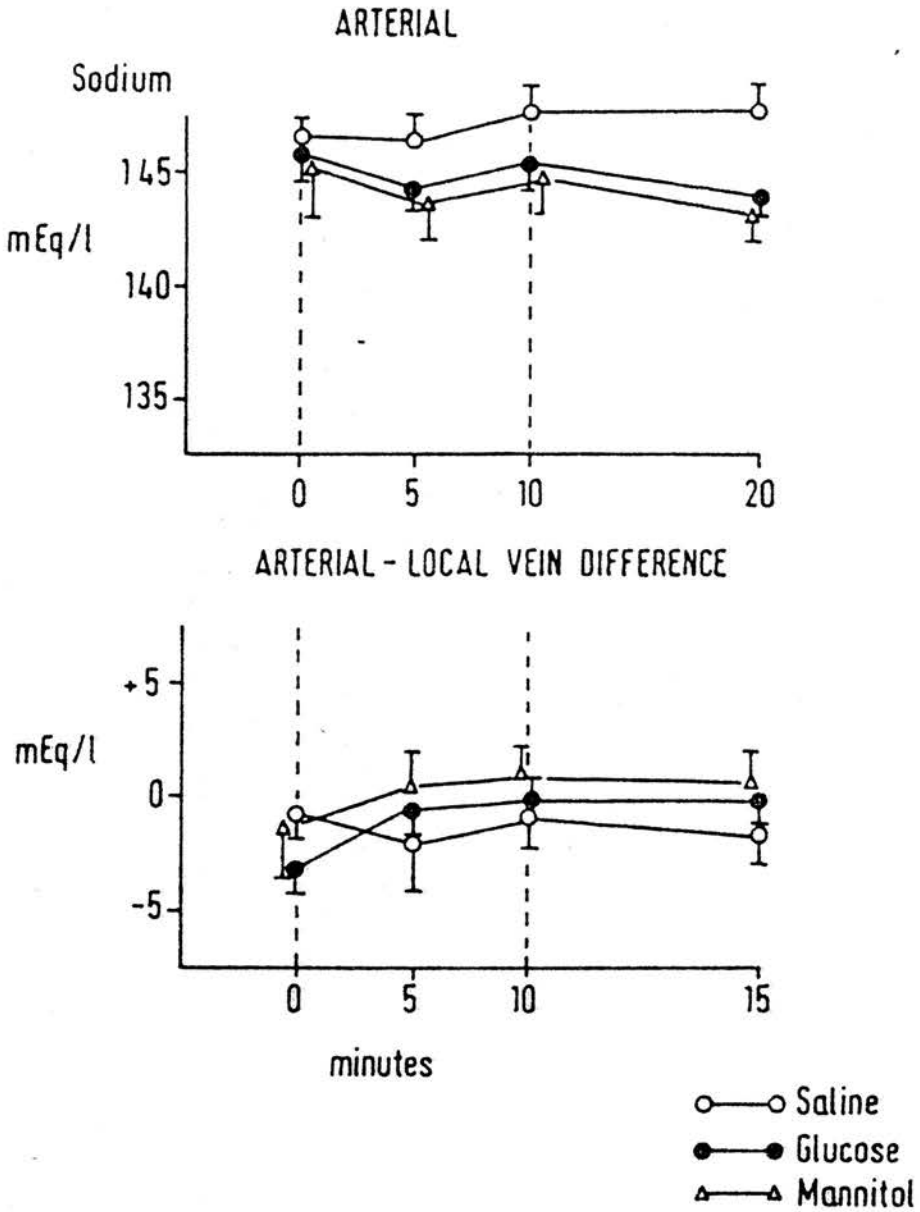


FIG. 31. Effect of saline, glucose and mannitol infusions on arterial and arterial-local venous differences of sodium following coronary occlusion. The small reduction in plasma sodium during glucose and mannitol infusion did not attain significance

Summary

1. No significant differences in VPBT occurred during 10 minute periods of moderate ischaemia between occlusions during saline or mannitol infusions.

2. Glucose infusion sufficient to approximately double arterial levels of glucose caused:-

   a) an increase in VPBT and decrease of VPB frequency apparent within two to seven minutes of occlusion.

   b) a significant increase in arterial-local venous difference of glucose concentrations, apparent before and within five minutes of arterial occlusion.

   c) a small fall in arterial potassium levels.

   d) no change in arterial-local venous differences of potassium, lactate or FFA.

3. The effects of glucose infusion were not reproduced by isosomotic mannitol infusion.

Effects of glucose on regional refractory periods

Mean values of RP change during coronary occlusion in eight dogs are shown in Table 13 and Fig. 32 under control conditions and during infusion of glucose. There were no significant changes in RP in the normal zone, either before, during or after coronary occlusion or during infusion of glucose.

As in earlier control studies, a variable response to ischaemia was observed. Of the eight dogs, in the central ischaemic area CA a progressive shortening of RP (5 dogs), shortening followed by prolongation (3 dogs) or prolongation of RP (1 dog) occurred during control occlusions. In the border zone progressive shortening (5 dogs), shortening followed by prolongation (1 dog) or prolongation (2 dogs) were seen. During infusion of glucose the alterations in patterns of change in RP during coronary occlusion were of:-

a) reduced shortening in RP (CA 5 dogs; PA 4 dogs)

b) prolongation of RP when previously shortening occurred (CA 1 dog; PA 1 dog)

c) increased prolongation of RP (CA 1 dog; PA 0 dogs)

d) no obvious change (CA 1 dog; PA 3 dogs)

Glucose infusion resulted in significant reduction in RP shortening at 7½ minutes after coronary occlusion, mean values being increased from 133.6 $\pm$ 5.1 to 143.1 $\pm$ 4.5 mS ($P < 0.05$), in CA. Similarly, RP was significantly prolonged at 5 minutes ($P < 0.05$) and 12½ minutes ($P < 0.01$) after occlusion in the PA during glucose infusion.

Gradients of refractoriness

The effect of glucose on mean gradients of refractoriness during the period of coronary occlusion is shown in Fig. 33 and Table 14.

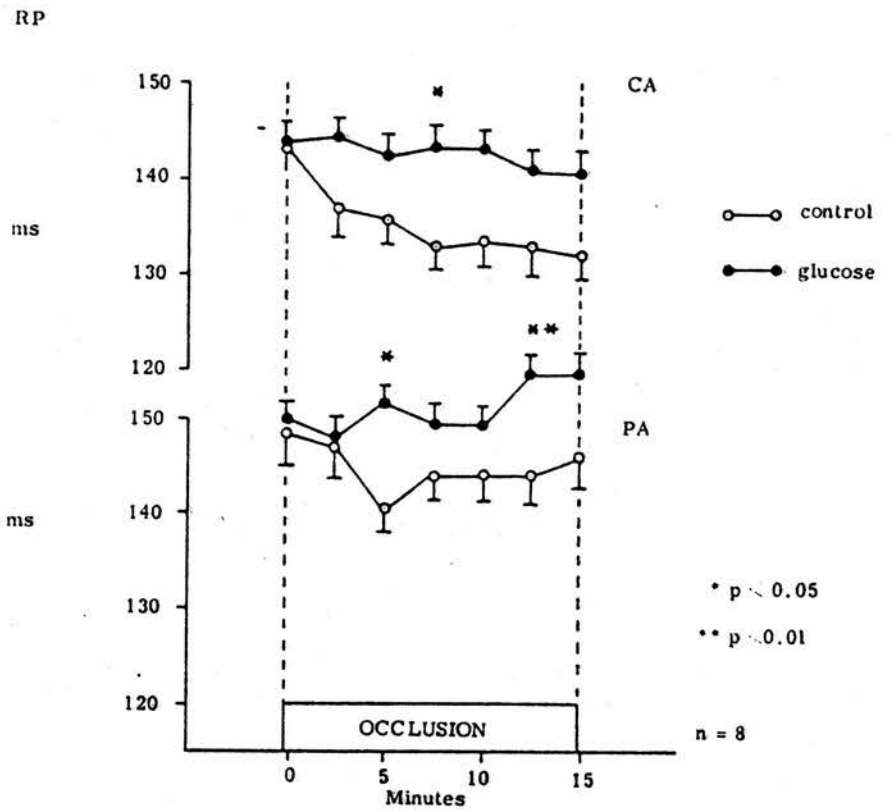


FIG. 32. Effect of glucose on regional ventricular refractory periods recorded in central (CA) and border (BA) ischaemia areas following coronary occlusion. Significant reduction of RP shortening occurred at 7½ minutes in CA and at 5 and 12½ after occlusion in BA. There were no changes in RP in the normal area. Mean values from 8 studies are shown

TABLE 13 Effect of infusion of glucose on ventricular refractoriness during acute coronary arterial occlusion in 8 dogs

| Minutes after occlusion | RP mS | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Glucose | | |
| | NA | CA | PA | NA | CA | PA |
| 0 | 145.1 ± 6.2 | 143.0 ± 6.4 | 148.3 ± 7.4 | 145.8 ± 6.7 | 144.3 ± 6.6 | 150.1 ± 7.8 |
| 2½ | 144.8 ± 5.8 | 137.0 ± 6.6 | 147.1 ± 10.1 | 145.2 ± 6.9 | 144.7 ± 6.4 | 147.6 ± 7.8 |
| 5 | 144.9 ± 6.0 | 135.9 ± 6.5 | 140.8 ± 7.9 | 145.3 ± 5.8 | 142.6 ± 4.6 | 153.7 ± 7.6* |
| 7½ | 145.2 ± 6.1 | 133.6 ± 5.1 | 144.3 ± 7.6 | 145.8 ± 6.2 | 143.1 ± 4.5* | 149.0 ± 6.7 |
| 10 | 144.7 ± 4.3 | 134.5 ± 6.6 | 144.1 ± 8.2 | 145.0 ± 6.3 | 143.6 ± 3.7 | 149.3 ± 7.1 |
| 12½ | 144.6 ± 4.6 | 133.8 ± 7.8 | 144.0 ± 7.9 | 145.3 ± 6.2 | 141.7 ± 4.0 | 155.2 ± 8.1** |
| 15 | 145.0 ± 5.2 | 132.5 ± 6.6 | 146.5 ± 10.0 | 146.1 ± 5.8 | 140.9 ± 4.3 | 155.1 ± 8.4 |

* $P < 0.05$

** $P < 0.01$

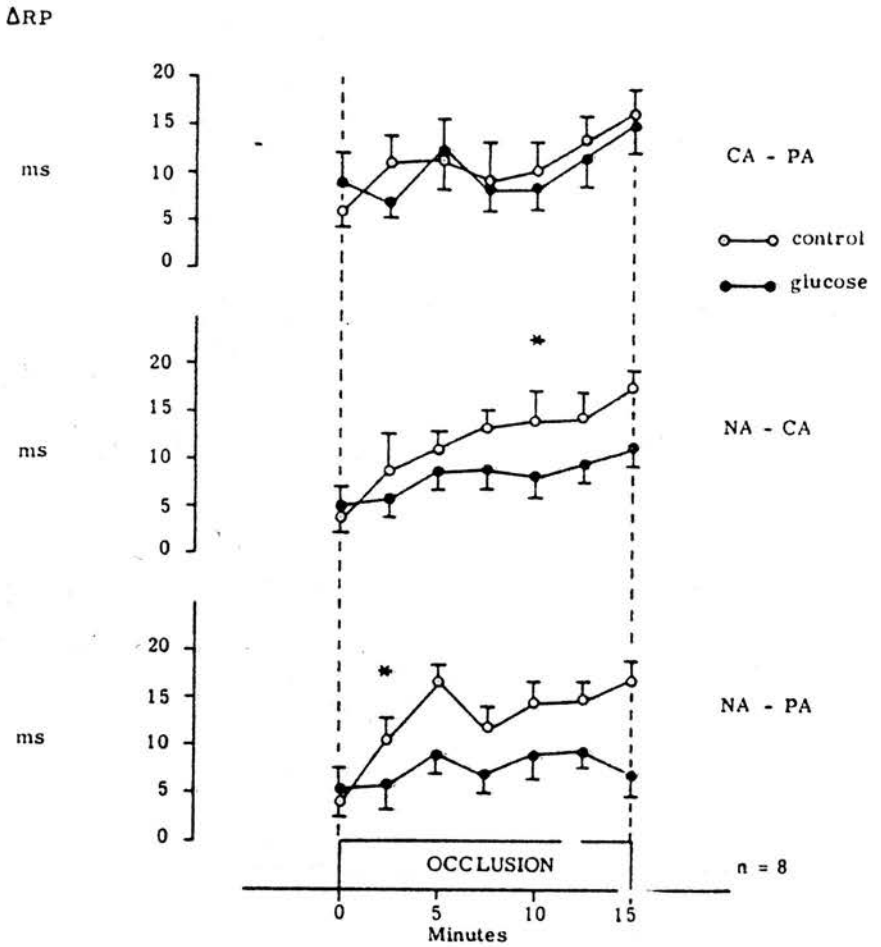


FIG. 33. Effect of glucose on gradients of refractoriness between normal (NA), central ischaemic (CA) and border ischaemic (PA) areas of myocardium following coronary occlusion in 8 dogs. No significant effects were observed within the ischaemic zone (CA - PA), but significant beneficial effects of glucose occurred at 2½ minutes (NA - PA) and 10 minutes (NA - CA) after occlusion between normal and ischaemic tissue

TABLE 14 Effect of infusion of glucose on ventricular gradients of refractoriness during acute coronary occlusion in 8 dogs

| Minutes after occlusion | Δ RP mS | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Glucose | | |
| | NA - CA | NA - PA | CA - PA | NA - CA | NA - PA | CA - PA |
| 0 | 5.8 ± 1.3 | 6.2 ± 1.8 | 5.3 ± 1.8 | 5.9 ± 1.5 | 6.1 ± 1.5 | 8.2 ± 2.3 |
| 2½ | 9.0 ± 4.0 | 10.5 ± 1.7 | 11.2 ± 2.5 | 6.1 ± 1.6 | 5.5 ± 1.9* | 7.3 ± 1.5 |
| 5 | 11.4 ± 4.3 | 17.2 ± 2.8 | 11.4 ± 2.8 | 8.5 ± 2.6 | 8.8 ± 2.0 | 12.5 ± 3.2 |
| 7½ | 12.8 ± 3.8 | 11.7 ± 3.0 | 9.8 ± 4.2 | 8.7 ± 2.0 | 7.0 ± 1.5 | 8.5 ± 2.9 |
| 10 | 13.5 ± 3.0 | 14.1 ± 2.8 | 10.3 ± 3.3 | 8.1 ± 2.1* | 9.2 ± 1.6 | 8.2 ± 2.7 |
| 12½ | 13.9 ± 3.9 | 14.7 ± 2.5 | 13.6 ± 4.6 | 9.8 ± 3.6 | 9.7 ± 1.9 | 14.6 ± 4.3 |
| 15 | 15.5 ± 5.7 | 17.0 ± 4.6 | 15.5 ± 5.2 | 11.5 ± 3.3 | 7.1 ± 1.2 | 15.2 ± 4.1 |

* $P < 0.05$

Gradients are shown between normal and central ischaemic areas (NA - CA), between normal and peripheral ischaemic areas (NA - PA) and across the ischaemic area (CA - PA). Gradients between normal and ischaemic tisue (NA - CA; NA - PA) were consistently reduced by glucose and attained significance ($P < 0.05$) at 2½ minutes (NA - PA) and 10 minutes (NA - CA) of ischaemia. No difference was apparent in gradients between central and peripheral ischaemic tissue (CA - PA). Pooling of data from NA - CA and NA - PA gradients revealed a significant reduction in refractory period gradients ($P < 0.05$) by glucose at 2½ minutes, 10 minutes and 12½ minutes after coronary occlusion.

Conduction delays

Endocardial-epicardial conduction time increased from 22.2 ± 3.0 and 21.2 ± 2.5 mS in CA and PA respectively to 30.0 ± 4.8 and 25.5 ± 2.1 mS after 15 minutes of coronary occlusion. During infusion of glucose conduction was not altered to CA (21.0 ± 3.2 mS) or PA (23.7 ± 2.5 mS) prior to occlusion. A slight reduction in delays was observed after 15 minutes of ischaemia (25.5 ± 2.1; 25.0 ± 2.2 mS in CA, PA), compared with the control occlusion, but this did not attain significant levels.

Metabolic data

Biochemical data obtained during control occlusions and during infusion of glucose are shown in Table 15.

Arterial plasma glucose concentrations were increased from 5.29 ± 0.35 to 16.70 ± 1.18 mM by infusion of glucose ($P < 0.001$), reaching steady state levels between 7½ and 15 minutes after coronary occlusion. The increase in arterial-local venous difference of

TABLE 15 Metabolic gradients (arterio-venous differences) across ischaemic myocardium during infusion of glucose in studies of ventricular refractoriness (8 dogs)

| | Minutes after occlusion | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Glucose | | |
| | 0 | 7½ | 15 | 0 | 7½ | 15 |
| **Glucose mM** | | | | | | |
| Arterial | 5.29 ± 0.35 | 5.33 ± 0.34 | 5.43 ± 0.30 | 16.70 ± 1.18 * | 18.95 ± 1.41 * | 19.17 ± 1.55 * |
| Local Venous | 4.83 ± 1.17 | 3.64 ± 0.27 | 4.29 ± 1.32 | 16.44 ± 2.54 * | 17.15 ± 3.08 * | 16.93 ± 3.71 * |
| A-1v | 0.70 ± | 1.72 ± 0.36 | 1.33 ± 0.27 | 0.80 ± 0.51 | 2.90 ± 1.31 | 3.20 ± 0.80 * |
| **Potassium mM** | | | | | | |
| Arterial | 3.95 ± 0.13 | 3.78 ± 0.10 | 3.68 ± 0.12 | 3.41 ± 0.08 * | 3.09 ± 0.08 * | 3.02 ± 0.09 * |
| Local Venous | Ins | Ins | Ins | Ins | Ins | Ins |
| A-1v | Ins | Ins | Ins | Ins | Ins | Ins |
| **Lactate mM** | | | | | | |
| Arterial | 1.14 ± 0.13 | 1.19 ± 0.12 | 1.14 ± 0.13 | 1.37 ± 0.07 * | 1.78 ± 0.16 * | 2.20 ± 0.26 * |
| Local Venous | 1.02 ± 0.11 | 4.16 ± 0.51 | 4.89 ± 0.84 | 1.44 ± 0.10 * | 4.08 ± 0.70 | 4.38 ± 0.74 |
| A-1v | 0.07 ± 0.14 | -3.06 ± 0.52 | -3.79 ± 0.82 | -0.06 ± 0.12 | -2.32 ± 0.62 | -2.16 ± 0.76 |
| **FFA μM** | | | | | | |
| Arterial | 735 ± 101 | 727 ± 103 | 768 ± 95 | 602 ± 45 | 505 ± 30 | 566 ± 39 |
| Local Venous | 648 ± 116 (3) | 620 ± 124 (3) | 702 ± 102 (3) | 457 ± 176 (3) | 502 ± 134 (3) | 475 ± 138 (3) |
| A-1v | 296 ± 55 (3) | 241 ± 83 (3) | 336 ± 44 (3) | 146 ± 31 (3) | 55 ± 61 (3) | 113 ± 75 (3) |

* $P < 0.05$

glucose during ischaemia was further increased by infusion of glucose, values at 15 minutes after occlusion being 1.33 ± 0.27 and 3.20 ± 0.80 mM ($P < 0.05$) respectively. The slightly higher arterial glucose levels in this series of studies was related to a slightly higher loading infusion rate of glucose (35 $mg.Kg^{-1}.min^{-1}$ for 5 minutes) than in the other studies (30 $mg.Kg^{-1}.min^{-1}$).

Release of lactate from the ischaemic zone resulted in negative arterial-local venous differences during both control and glucose treated occlusions, but values did not differ significantly. A small increase in arterial lactate concentrations was observed during the glucose infusion ($P < 0.01$).

Arterial free fatty acid concentrations were slightly, but not significantly, reduced by glucose infusion. Arterial local venous differences of FFA were similarly reduced both before and during ischaemia by glucose infusion, but insufficient sampling precluded statistical analysis.

Arterial concentrations of potassium were reduced from 3.95 ± 0.13 to 3.41 ± 0.08 mM ($P < 0.05$) during glucose infusion.

Regional myocardial blood flow

Glucose infusion did not result in any significant change in regional blood flow in either normal or ischaemic myocardium (Table 16). Endocardial-epicardial blood flow ratios in the ischaemic zone were unaffected (0.69 ± 0.12, 0.69 ± 0.08 respectively). The mean reduction in blood flow in the ischaemic area was slightly less in the endocardium after glucose (from 34 to 41% normal), but these changes did not attain significance. Epicardial flows were unaffected (from 60% to 58% normal after glucose) during ischaemia.

TABLE 16 Effect of glucose on regional myocardial blood flow following coronary occlusion during studies of regional refractoriness in 5 dogs. Mean values ± S.E.M. are shown.

| | Regional Myocardial Blood Flow $ml.g^{-1}.min^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Glucose | | |
| | Endo | Epi | Endo/Epi | Endo | Epi | Endo/Epi |
| Normal zone | 0.79 ± 0.03 | 0.70 ± 0.05 | 0.92 ± 0.06 | 0.75 ± 0.10 | 0.77 ± 0.10 | 1.00 ± 0.06 |
| Ischaemic zone | 0.27 ± 0.04 | 0.42 ± 0.12 | 0.69 ± 0.12 | 0.31 ± 0.08 | 0.45 ± 0.14 | 0.69 ± 0.06 |

Effect of glucose after one hour of ischaemia

The effects of glucose infusion on RP and epicardial conduction time determined in normal and ischamic (CA) myocardium after one hour of coronary occlusion producing "mild" ischaemia and RP shortening are shown for five dogs in Table 17.

Steady levels of RP and of the difference in epicardial activation time between NA and CA were obtained between 30 and 60 minutes of ischaemia. Following infusion of glucose there was an increase in RP, evident within one minute in some dogs to almost control values (NA). RP in CA rose from 129 $\pm$ 4 mS immediately before glucose infusion to 145 $\pm$ 6 mS after 15 minutes, which did not differ significantly from RP in NA (148 $\pm$ 3 mS). Arterial glucose at this time rose from 5.3 $\pm$ 0.3 mM to 14.2 $\pm$ 1.2 mM.

Control studies of RP between 60 and 120 minutes after coronary occlusion in two dogs did not show similar directional changes in RP at this time.

On discontinuing glucose infusion a decline in RP towards original values began between 15 and 25 minutes later. RP fell to 136 $\pm$ 6 mS at 120 minutes of ischaemia.

A small, but not significant, improvement in ischaemic conduction occurred at this time.

TABLE 17 Effect of glucose infusion on ventricular refractoriness and epicardial conduction time after 60 minutes of ischaemia in 5 dogs

| Minutes after occlusion | RP mS | | CT mS | Arterial Glucose mM |
|---|---|---|---|---|
| | NA | CA | CA - NA | |
| 30 | 148.4 ± 2.8 | 122.6 ± 1.0† | 19.5 ± 2.3 | |
| 35 | 148.1 ± 3.0 | 123.4 ± 2.0† | 19.4 ± 2.3 | |
| 40 | 147.8 ± 3.2 | 124.6 ± 3.0† | 19.4 ± 2.4 | |
| 45 | 147.9 ± 3.2 | 125.0 ± 3.3† | 19.4 ± 2.4 | |
| 50 | 148.8 ± 2.9 | 125.0 ± 2.8† | 19.4 ± 2.4 | |
| 55 | 148.7 ± 2.9 | 126.2 ± 2.9† | 19.4 ± 2.4 | 5.3 ± 0.3 |
| 60 | 148.6 ± 2.9 | 129.0 ± 4.3† | 19.4 ± 2.4 | |
| (Glucose commenced) | | | | |
| 65 | 148.4 ± 3.1 | 139.0 ± 6.1 | 19.8 ± 2.8 | |
| 70 | 148.6 ± 3.0 | 141.0 ± 5.8 | 19.7 ± 2.8 | |
| 75 | 148.4 ± 3.0 | 145.2 ± 6.0 | 19.7 ± 2.8 | 14.2 ± 1.2 |
| (Glucose discontinued) | | | | |
| 80 | 149.6 ± 2.9 | 141.4 ± 5.5 | 19.6 ± 2.8 | |
| 85 | 149.4 ± 3.0 | 146.4 ± 6.3 | 18.2 ± 2.5 | |
| 90 | 148.4 ± 3.1 | 145.6 ± 6.1 | 16.0 ± 2.5 | |
| 95 | 148.8 ± 3.1 | 140.0 ± 7.9 | 15.9 ± 2.6 | |
| 100 | 149.4 ± 2.9 | 141.0 ± 7.3 | 16.4 ± 2.7 | |
| 105 | 149.8 ± 2.4 | 139.6 ± 6.9 | 16.4 ± 2.7 | |
| 110 | 149.6 ± 2.5 | 138.6 ± 6.6 | 16.2 ± 2.8 | |
| 115 | 148.8 ± 3.0 | 137.2 ± 6.6 | 16.2 ± 2.8 | |
| 120 | 148.8 ± 3.0 | 136.0 ± 6.5 | 15.2 ± 3.2 | |

†$P < 0.01$

Summary

1. Glucose infusion significantly reduced the shortening of refractoriness after 7½ minutes of ischaemia in CA and after five and 12½ minutes of ischaemia in PA.

2. Glucose infusion did not affect gradients of refractoriness in the ischaemic zone (CA - PA). A significant reduction in gradients between normal and ischaemic area (NA - CA + NA - PA) occurred at 2½, 10 and 12½ minutes after occlusion.

3. Small conduction delays induced by ischaemia were not significantly reduced by glucose.

4. Associated metabolic effects of glucose included an approximate doubling of arterial glucose levels, a small reduction in arterial potassium, and an increase in arterio-venous differences of glucose.

5. Regional myocardial blood flow was not influenced by glucose infusion.

6. After 60 minutes of ischaemia glucose infusion was effective in prolonging shortened refractoriness. Small improvements in conduction did not attain significance.

Effect of glucose on potential changes during acute ischaemia

High coronary occlusion

Mean values of APD and CT following high coronary occlusion are shown in Fig. 34 and tabulated in Table 18 from studies in 11 dogs under control conditions and during infusion of glucose. The incidence of VF before and after glucose is also shown.

Rapid changes in APD and CT occurred during control occlusions between one and three minutes of ischaemia with shortening of APD from 185 ± 8 mS to 138 ± 6 mS. Loss of AP amplitude and reduction of initial upstroke velocity was noted, but not quantitated. Endocardial-epicardial CT prolonged from 24 ± 1 mS to 46 ± 6 mS over this period.

VF resulted in five of 11 animals within the five minute occlusion period.

Following infusion of glucose rapid changes in APD and CT again followed coronary occlusion between one and three minutes of ischaemia. APD shortening was transiently delayed, however, so that APD was significantly less shortened ($P < 0.05$) after glucose (187 ± 10 mS) than control (153 ± 8 mS) at 1½ minutes of ischaemia. Similarly, endocardial-epicardial CT was reduced significantly ($P < 0.05$) at 1½ minutes of ischaemia from 37 ± 5 to 28 ± 2 mS after glucose. No significant differences were found subsequently.

The incidence of timing of VF after glucose was not significantly different from control. Six dogs developed VF during a glucose treated occlusion, compared with five dogs during a control occlusion.

ST-segment analysis was not meaningful in this group of animals on account of the severity of ischaemia and distortion of the ST-segment by delayed depolarisations.

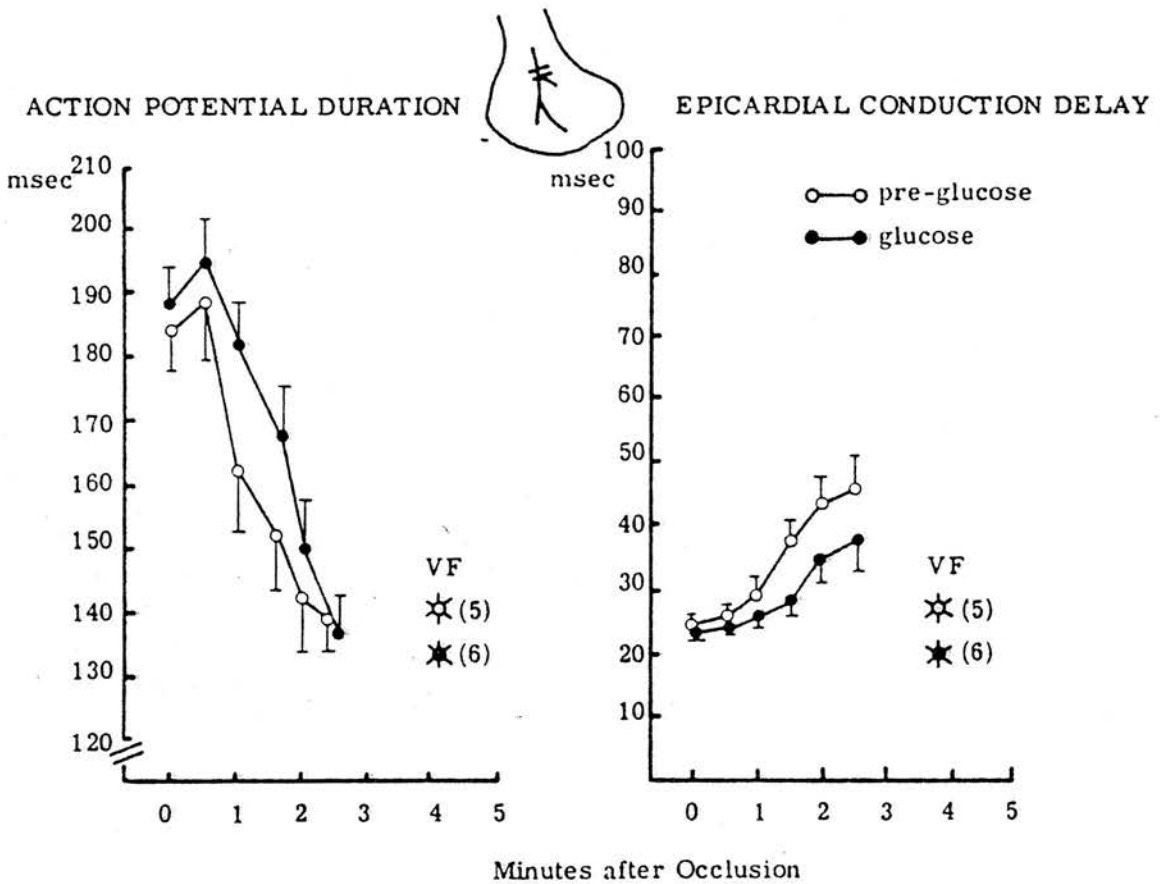


FIG. 34. Effect of glucose on action potential duration and endocardial-epicardial conduction delay following high (proximal) occlusion of the left anterior descending coronary artery in 11 dogs. The incidence of ventricular fibrillation was not influenced by glucose administration. Transient beneficial effects on action potential duration and conduction delay were observed at 1½ and 2 minutes, and 1½ minutes after occlusion respectively

BLE 18 Effect of glucose infusion on action potential duration, endocardial-epicardial conduction time and incidence of ventricular fibrillation following high coronary occlusion in 11 dogs

| Time after occlusion | APD mS | | CT mS | | VF | |
|---|---|---|---|---|---|---|
| | Control | Glucose | Control | Glucose | Control | Glucose |
| 0 | 185 ± 8 | 188 ± 7 | 24 ± 1 | 24 ± 1 | - | - |
| ½ | 188 ± 10 | 195 ± 10 | 25 ± 1 | 24 ± 1 | - | - |
| 1 | 164 ± 8 | 182 ± 11 | 29 ± 3 | 25 ± 1 | - | - |
| 1½ | 153 ± 8 | 167 ± 10* | 37 ± 5 | 28 ± 2* | - | - |
| 2 | 143 ± 8 | 151 ± 9* | 43 ± 15 | 35 ± 5 | - | - |
| 2½ | 138 ± 6 | 139 ± 11 | 46 ± 6 | 35 ± 6 | - | - |
| 3 | | | | | ++ | +++ |
| 3½ | | | | | | + |
| 4 | | | | | ++ | + |
| 4½ | | | | | + | + |
| 5 | | | | | | |

P < 0.05

episode of VF in one dog

In view of the early onset of VF in this group of animals metabolic data was restricted to measurement of arterial levels of substrates. Determination of local venous biochemistry or regional myocardial blood flow was not possible.

Arterial glucose levels were elevated from 5.1 ± 0.14 mM during control occlusions to 12.5 ± 1.8 mM during infusion of glucose. Plasma FFA showed little change from 724 ± 82 before control occlusion to 689 ± 67 $\mu Eq.l^{-1}$ during infusion of glucose.

Low coronary occlusion

Less severe ischaemic changes were induced in seven dogs with a lower coronary occlusion. None of these animals developed VF during the five minute occlusion period, nor did any arrhythmia appear. Preocclusion APD was slightly less than in the high occlusion group. Changes in APD and CT are shown in Fig. 35 and in Table 19. Changes in APD were significantly less from ½ minute after coronary occlusion ($P < 0.001$), from 1 to 3 minutes ($P < 0.001$), 4 to 4½ minutes ($P < 0.01$) and 5 minutes ($P < 0.05$) after occlusion. The prolongation of conduction was delayed compared with the high occlusion group and almost completely abolished by glucose. Significant reductions were found at 2½, 4½ and 5 minutes after occlusion.

Mean ST-segment elevation was 9.1 ± 1.2 mV before and 6.9 ± 1.2 mV ($P < 0.025$) at 5 minutes of ischaemia after glucose administration.

No significant differences in APD, CT or mean ST-segment elevation occurred between occlusions before and after glucose administration before coronary occlusion.

No significant differences between control occlusions before and after discontinuing glucose were found for APD or CT.

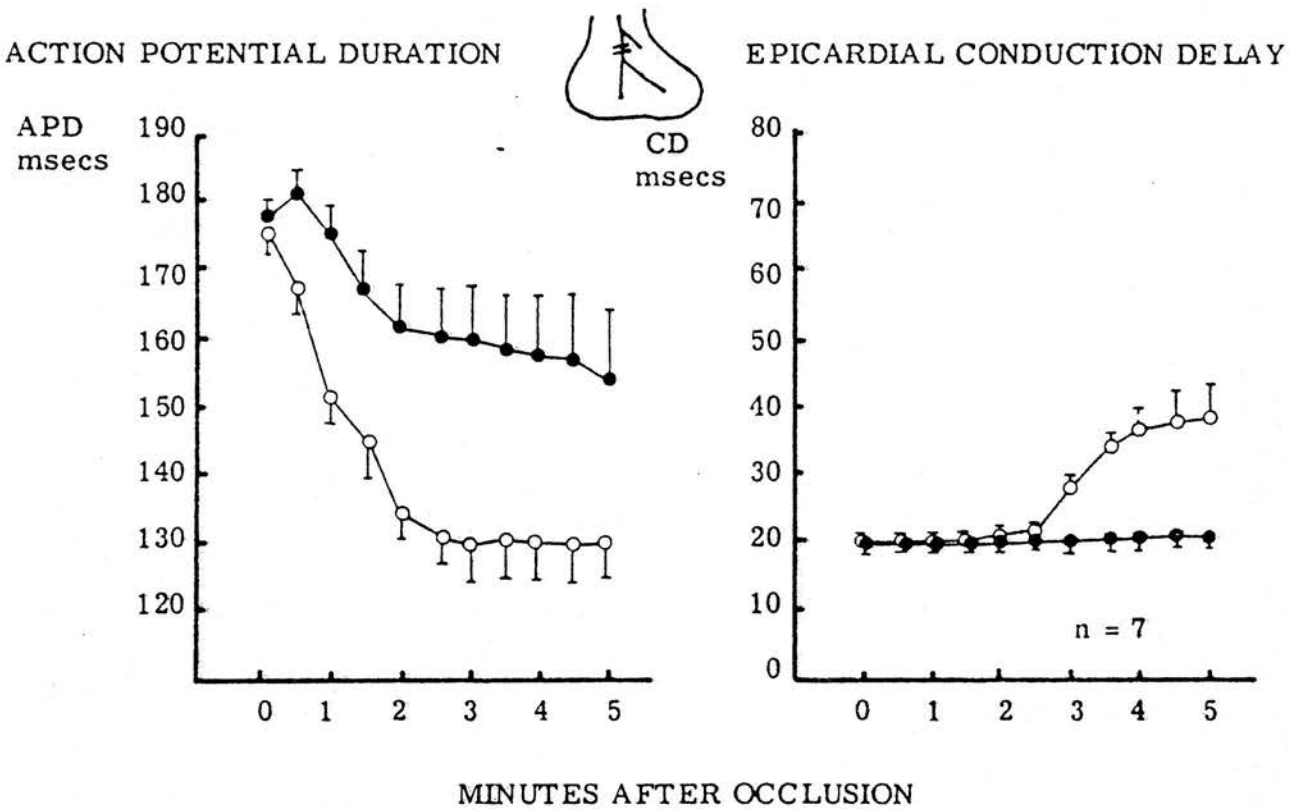


FIG. 35. Effect of glucose on action potential duration and endocardial-epicardial conduction delay following low (distal) coronary occlusion in 7 dogs. Significant improvement in APD was observed from ½ minute of occlusion and near normalisation of conduction changes

TABLE 19 Effect of glucose on action potential duration and endocardial-epicardial conduction time following low coronary artery occlusion in 7 dogs

| Time after occlusion | APD mS | | CT mS | |
|---|---|---|---|---|
| | Control | Glucose | Control | Glucose |
| 0 | 175 ± 1 | 178 ± 3 | 21 ± 1 | 21 ± 1 |
| ½ | 167 ± 3 | 181 ± 4*** | 21 ± 1 | 21 ± 1 |
| 1 | 154 ± 4 | 176 ± 5*** | 21 ± 1 | 21 ± 1 |
| 1½ | 144 ± 4 | 168 ± 6*** | 22 ± 1 | 21 ± 1 |
| 2 | 146 ± 4 | 163 ± 7*** | 23 ± 1 | 22 ± 1 |
| 2½ | 131 ± 4 | 161 ± 7*** | 24 ± 1 | 22 ± 1* |
| 3 | 129 ± 8 | 159 ± 9*** | 29 ± 4 | 23 ± 1 |
| 3½ | 130 ± 8 | 158 ± 9** | 35 ± 8 | 23 ± 1 |
| 4 | 130 ± 7 | 157 ± 15** | 36 ± 8 | 23 ± 1 |
| 4½ | 129 ± 8 | 158 ± 15** | 37 ± 8 | 23 ± 1* |
| 5 | 129 ± 8 | 154 ± 15** | 38 ± 9 | 23 ± 1* |

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

Metaboiic findings

Metabolic data was obtained in this series of animals as no dog developed VF and local venous effluent flow was greater than in the high occlusion group. Arterial levels and metabolic gradients of glucose, FFA, lactate and potassium are shown in Fig. 36 and in Table 20.

Arterial levels of glucose were approximately doubled from control (4.9 ± 0.13 mM) to 13.2 ± 1.6 mM ($P < 0.001$) after infusion of glucose. The arterial local venous differences of glucose were increased both before occlusion from 0.34 ± 0.16 mM to 0.93 ± 0.11 mM ($P < 0.05$), and after 5 minutes of ischaemia from 0.75 ± 0.14 mM to 1.89 ± 0.62 mM.

A small increase in arterial lactate was observed during glucose infusion. During ischaemia an excess of local venous over arterial levels of lactate resulted in a negative arterial-local venous difference for lactate. Glucose did not influence this gradient, however, during occlusion.

A significant fall ($P < 0.05$) in arterial potassium followed glucose infusion, although small in magnitude (3.8 ± 0.115 to 3.3 ± 0.15 mM). Again an excess of local venous over arterial potassium levels during ischaemia resulted in negative gradients during ischaemia. These gradients were not influenced by glucose administration.

Glucose had no significant effect on arterial levels of plasma FFA or on metabolic gradients of FFA before or during ischaemia.

Regional myocardial blood flow

Regional myocardial blood flow was determined in both non-ischaemic and ischaemic myocardium following low coronary occlusion.

TABLE 20 Effect of glucose on metabolic gradients across ischaemic myocardium during electrophysiological studies of low coronary occlusion in 7 dogs

| | Minutes after occlusion | | | |
|---|---|---|---|---|
| | Control | | Glucose | |
| | 0 | 5 | 0 | 5 |
| Glucose mM | | | | |
| Arterial | 4.9 ± 0.13 | 5.2 ± 0.16 | 13.2 ± 1.6*** | 12.3 ± 2.1*** |
| Local venous | 4.9 ± 0.12 | 4.8 ± 0.16 | 12.1 ± 1.6*** | 11.0 ± 1.5*** |
| A-lv | 0.34 ± 0.16 | 0.75 ± 0.14 | 0.93 ± 0.11 | 1.89 ± 0.62* |
| Lactate mM | | | | |
| Arterial | 0.73 ± 0.13 | 0.63 ± 0.16 | 1.03 ± 0.12 | 1.13 ± 0.11 |
| Local venous | 0.62 ± 0.13 | 2.38 ± 0.13 | 0.82 ± 0.13 | 2.35 ± 0.23 |
| A-lv | +0.26 ± 0.15 | -1.44 ± 0.19 | +0.18 ± 0.09 | -1.23 ± 0.22 |
| Potassium mM | | | | |
| Arterial | 3.8 ± 0.16 | 3.8 ± 0.16 | 3.3 ± 0.15* | 3.2 ± 0.17* |
| Local venous | 3.6 ± 0.21 | 3.8 ± 0.21 | 3.4 ± 0.05* | 3.7 ± 0.17 |
| A-lv | 0.16 ± 0.15 | -0.02 ± 0.54 | -0.25 ± 0.12 | -0.47 ± 0.20 |
| Free fatty acid μM | | | | |
| Arterial | 681 ± 82 | 679 ± 65 | 816 ± 95 | 712 ± 92 |
| Local venous | 429 ± 62 | 452 ± 47 | 509 ± 51 | 450 ± 52 |
| A-lv | 251 ± 90 | 223 ± 63 | 306 ± 74 | 271 ± 70 |

*$P < 0.05$ **$P < 0.02$ ***$P < 0.001$

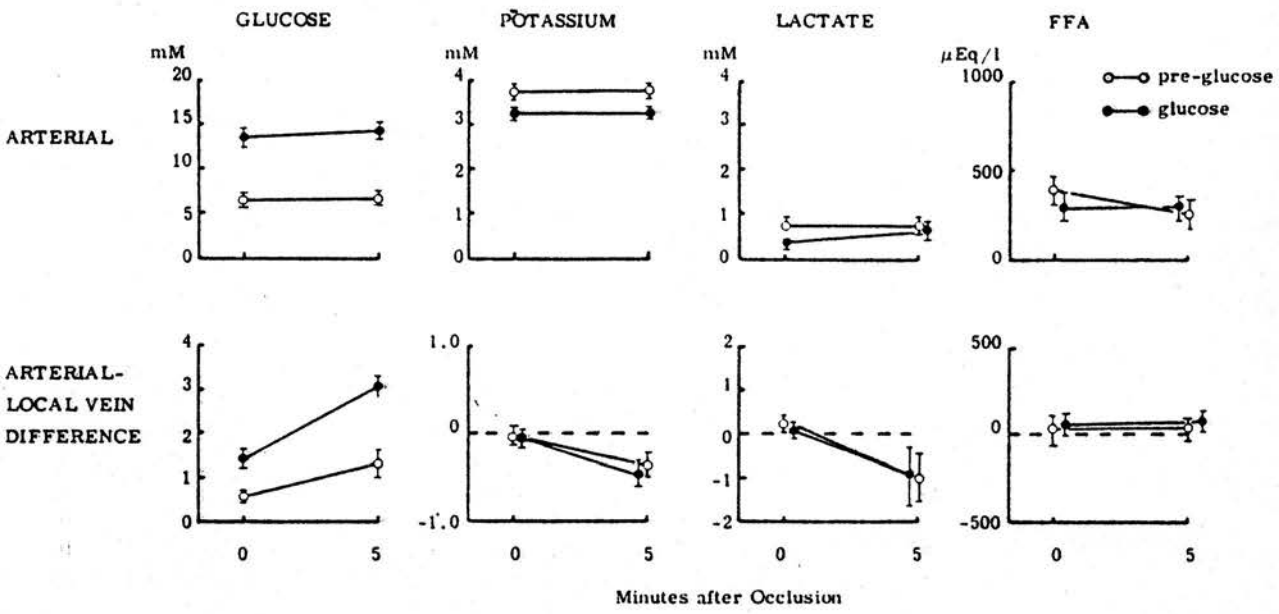


FIG. 36. Effect of glucose on arterial and arterial-local venous differences of glucose, potassium, lactate and FFA following low (distal) coronary occlusion

Absolute flow values during the control occlusion and during infusion of glucose are shown in Table 21.

After 3½ minutes of ischaemia flow in the ischaemic zone was reduced to 47% and 62% of that in the normal zone in endocardial and epicardial samples respectively. The mean endocardial:epicardial blood flow ratio was reduced from 1.10 to 0.81. The distribution of regional myocardial blood flow during ischaemia was not significantly altered during infusion of glucose. Thus, blood flow in the ischaemic zone was 41% and 71% of that in the normal area in endocardial and epicardial samples respectively, and the mean endocardial:epicardial blood flow ratio was reduced from 1.10 to 0.73.

Blood flows during the first and second control occlusions did not differ significantly.

TABLE 21 Effect of glucose infusion on regional myocardial blood flow determined 3½ minutes after low coronary occlusion in 5 dogs

| | Regional Myocardial Blood Flow ml.g-1.min-1 | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Glucose | | |
| | Endo | Epi | Endo/Epi | Endo | Epi | Endo/Epi |
| Normal Zone | 1.08 ± 0.03 | 1.04 ± 0.04 | 1.10 ± 0.01 | 1.114 ± 0.07 | 1.05 ± 0.06 | 1.10 ± 0.01 |
| Ischaemic Zone | 0.51 ± 0.02 | 0.65 ± 0.02 | 0.81 ± 0.03 | 0.52 ± 0.04 | 0.75 ± 0.06 | 0.73 ± 0.03 |

Summary

Glucose infusion sufficient to approximately double arterial levels of glucose had the following effects following acute coronary occlusion.

1. Transient delay in APD shortening and transient reduction of CT prolongation (high coronary occlusion).

2. No effect on APD, CT or incidence of VF between 3 and 5 minutes (high coronary occlusion).

3. Significant reduction in APD shortening between ½ minute and 5 minutes (low coronary occlusion).

4. Significant reduction in CT prolongation at 2½, 4½ and 5 minutes (low coronary occlusion).

5. Significant increase in arterial-local venous gradient of glucose (low coronary occlusion).

6. Significant reduction in arterial potassium levels.

7. No change in metabolic gradients (A-lv difference) of lactate, potassium of FFA.

8. No change in regional myocardial blood flow of either normal or ischaemic myocardium or endocardium or epicardium (low coronary occlusion).

## 11. ELECTROPHYSIOLOGICAL EFFECTS OF ANTILIPOLYTIC THERAPY DURING ACUTE MYOCARDIAL ISCHAEMIA

### Effect of isoprenaline on VPBT

The effect of isoprenaline infusion on VPBT is illustrated in Fig. 37 and values shown in Table 22.

Mean VPBT in seven dogs was reduced from 24.3 ± 1.6 mA to 17.0 mA by infusion of isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ (n = 7) and to 19.2 ± 8.7 mA (n = 4) by the lower infusion concentration of 0.01 $\mu g.Kg^{-1}.min^{-1}$. (Studies were all at constant heart rate of 200 beats $min^{-1}$).

The fall in VPBT during ischaemia was more marked during isoprenaline. Mean VPBT after 5 minutes ischaemia was 17.9 ± 5.8 mA (control), compared with 4.5 ± 2.6 mA (low dose isoprenaline) and 1.3 mA (high dose isoprenaline, 0.1 $\mu g.Kg^{-1}.min^{-1}$).

### Effect of inhibition of lipolysis on VPBT

The effect of combined administration of isoprenaline and SAB 515, 5-fluoronicotinic acid, on VPBT, before and during ischaemia, is shown in Fig. 37 and in Table 22. Again SAB 515 was given in combination with low dose isoprenaline 0.01 $\mu g.Kg^{-1}.min^{-1}$ (n = 4) or high dose isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ of constant paced heart rate.

Higher values of VPBT were obtained before occlusion with combined SAB 515 and isoprenaline infusion, compared with isoprenaline infusion alone.

After five minutes of ischaemia no discernable beneficial effect of SAB 515 on VPBT was noted in any dog at either infusion rate of isoprenaline. Mean VPBT at this time was altered after SAB 515 from 4.5 ± 2.6 mA to 3.5 ± 1.7 mA at the low infusion rate of isoprenaline, and from 1.3 to 0 mA at the high infusion rate.

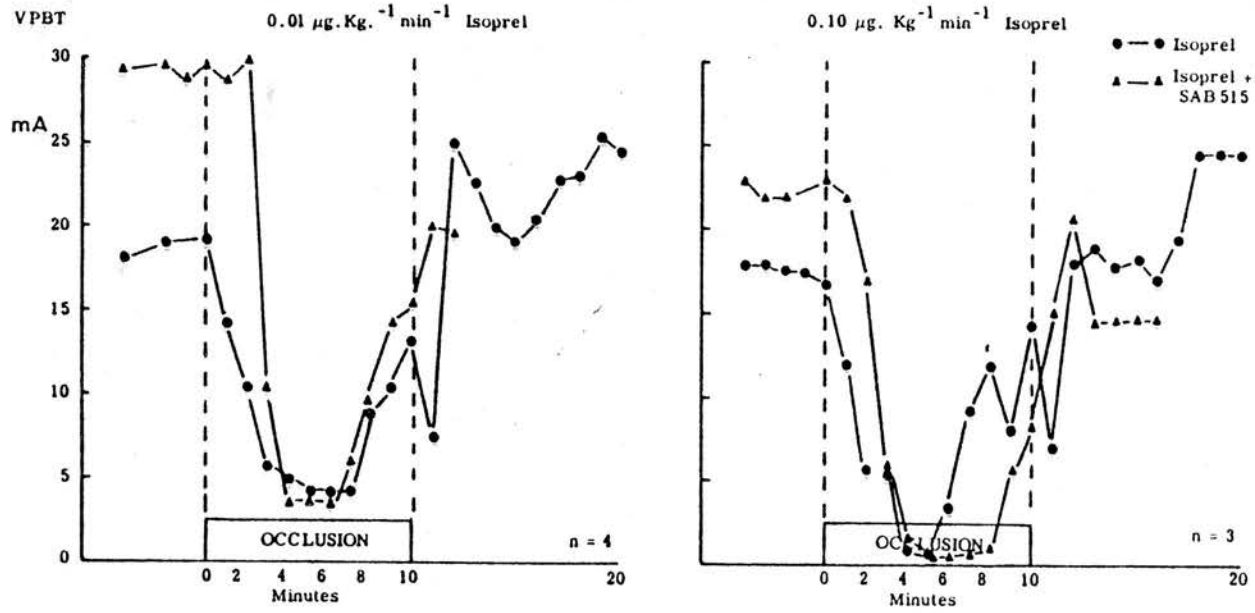


FIG. 37. Effect of inhibition of isoprenaline stimulated lipolysis with 5-fluoronicotinic acid (SAB 515) on VPBT following coronary occlusion. Mean values are shown during isoprenaline infusion at 0.01 $\mu g.Kg^{-1}.min^{-1}$ (n = 4) and 0.10 $\mu g.Kg^{-1}.min^{-1}$ (n = 3). No consistent beneficial effect was observed in any dog

TABLE 22 Effect of isoprenaline infusion on VPBT during 10 minute periods of coronary occlusion in 7 dogs

| Minutes after occlusion | VPBThreshold mA ± S.E.M. | | | | |
|---|---|---|---|---|---|
| | Control (n = 7) | Isoprel 0.01 $\mu g.Kg^{-1}.min^{-1}$ (n = 4) | Isoprel 0.01 $\mu g.Kg^{-1}.min^{-1}$ + SAB 5.15 0.5 $mg.Kg^{-1}.min^{-1}$ (n = 4) | Isoprel 0.1 $\mu g.Kg^{-1}.min^{-1}$ (n = 3) mean values only | Isoprel 0.1 $\mu g.Kg^{-1}.min^{-1}$ + SAB 5.5 0.5 $mg.Kg^{-1}.min^{-1}$ (n = 3) mean values only |
| 0 | 24.3 ± 1.6 | 19.2 ± 8.7 | 29.7 ± 9.4 | 17.0 | 23.0 |
| 1 | 25.1 ± 2.4 | 14.2 ± 6.5 | 29.2 ± 9.0 | 12.3 | 22.3 |
| 2 | 15.7 ± 1.6 | 11.7 ± 5.7 | 30.2 ± 8.5 | 6.6 | 17.3 |
| 3 | 13.6 ± 0.8 | 6.7 ± 4.1 | 11.0 ± 4.6 | 5.0 | 4.6 |
| 4 | 20.2 ± 3.8 | 5.7 ± 3.3 | 3.7 ± 1.3 | 1.5 | 1.6 |
| 5 | 17.9 ± 5.8 | 4.5 ± 2.6 | 3.5 ± 1.7 | 1.3 | 0 |
| 6 | 20.9 ± 2.3 | 4.5 ± 2.6 | 4.0 ± 2.2 | 3.0 | 0 |
| 7 | 23.8 ± 3.2 | 4.5 ± 2.2 | 3.0 ± 5.2 | 9.6 | 0 |
| 8 | 25.1 ± 5.5 | 8.7 ± 5.5 | 9.0 ± 5.8 | 12.3 | 1.0 |
| 9 | 25.8 ± 6.3 | 11.5 ± 6.6 | 14.0 ± 8.3 | 3.6 | 7.0 |
| 10 | 28.2 ± 6.2 | 13.2 ± 7.2 | 15.5 ± 9.3 | 14.0 | 8.7 |

In view of these negative findings further studies were not continued.

Metabolic findings in these studies are shown in Table 23. Isoprenaline at 0.1 $\mu g.Kg^{-1}.min^{-1}$ elevated arterial FFA to a mean value of 920 $\mu Eq.l^{-1}$, which was lowered to 4.33 $\mu Eq.l^{-1}$ by addition of 5-fluoronicotinic acid. Similarly, arterial glucose was elevated at 7.4 mM and lowered to 6.5 mM after 5-fluoronicotinic acid. At an infusion rate of 0.01 $\mu g.Kg^{-1}.min^{-1}$ isoprenaline there was minimal effect on arterial FFA levels, being 376 $\mu Eq.l^{-1}$, lowering to 220 $\mu Eq.l^{-1}$ after anti-lipolytic therapy.

During ischaemia negative arterial-local venous differences developed of lactate and potassium. No general trend was observed in observations following anti-lipolytic therapy.

Metabolic gradients were only determined at the low isoprenaline infusion rate of 0.01 $\mu g.Kg^{-1}.min^{-1}$.

TABLE 23 Metabolic gradients (arterial - local-venous differences) across ischaemic myocardium during infusion of isoprenaline and isoprenaline + 5-fluro-nicotinic acid. Mean values shown.

| | Minutes after occlusion | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ (n = 3) | | | I + SAB 515 | | | Isoprenaline 0.01 $\mu g.Kg^{-1}.min^{-1}$ (n = 4) | | | I + SAB 515 | | |
| | 0 | 5 | 10 | 0 | 5 | 10 | 0 | 5 | 10 | 0 | 5 | 10 |
| Glucose mM | | | | | | | | | | | | |
| Arterial | 7.4 | 7.7 | 7.5 | 6.5 | 6.8 | 6.9 | 5.8 | 5.8 | 5.6 | 5.0 | 4.8 | 4.8 |
| A-1v | Ins | Ins | Ins | Ins | Ins | Ins | Ins | Ins | Ins | Ins | Ins | Ins |
| FFA $\mu Eq/l$ | | | | | | | | | | | | |
| Arterial | 920 | 933 | 830 | 433 | 367 | 407 | 376 | 300 | 335 | 220 | 223 | 282 |
| A-1v | Ins | Ins | Ins | Ins | Ins | Ins | 17 | -57 | -70 | -67 | -10 | -80 |
| Lactate mM | | | | | | | | | | | | |
| Arterial | - | - | - | - | - | - | 1.27 | 1.30 | 1.28 | 1.39 | 1.32 | 1.42 |
| A-1v | - | - | - | - | - | - | -0.02 | -1.67 | -2.48 | 0.05 | -1.08 | Ins |
| Potassium mM | | | | | | | | | | | | |
| Arterial | 3.4 | 3.2 | 3.3 | 3.3 | 3.0 | 3.1 | 3.3 | 3.2 | 3.2 | 3.1 | 3.2 | 3.0 |
| A-1v | Ins | Ins | Ins | Ins | Ins | Ins | 0.02 | -0.35 | -0.68 | -0.05 | -0.48 | -0.76 |

Ins = Insufficient sample

Summary

1. VPBT was reduced by isoprenaline at high and low infusion rates (0.1 and 0.01 $\mu g.Kg^{-1}.min^{-1}$) before coronary occlusion.

2. In a limited series no effect was apparent of the addition of the antilipolytic agent 5-fluoronicotinic acid on VPBT during ischaemia at high or low infusion rates of isoprenaline.

3. An adequate lipolytic response was only obtained at the high dose of isoprenaline. Minimal antilipolytic effect was achieved therefore at the low infusion rate of isoprenaline.

Effects on regional ventricular refractoriness

Effect of isoprenaline infusion

Isoprenaline infusion 0.1 $\mu g.Kg^{-1}.min^{-1}$ over the period of the second test occlusion, at a constant heart rate of 200 beats $min^{-1}$, equal to that during the first occlusion, produced a significant fall in mean FRP ($P < 0.02$) of 8 mS in the non-ischaemic zone. Similarly, falls were observed in CA and PA (5 mS and 6 mS respectively).

Following coronary occlusion similar, but more rapid directional changes in FRP, occurred as in the control occlusions. A representative example is shown in Fig. 38. Shortening in FRP was more rapid in the ischaemic zone and greater than control. This shortening of FRP was followed by an earlier lengthening of FRP in the central area compared with control at a time when FRP in the peripheral area is still falling. This situation of enhanced divergence of adjacent FRP led to an increase in the gradient $\Delta$RP during coronary occlusion.

Mean values of FRP from nine dogs in NA, CA and PA under control conditions and during infusion of isoprenaline are shown in Fig. 39 and tabulated in Table 24. FRP remained significantly less than control ($P < 0.02$) in the non-ischaemic zone during occlusion. In the central ischaemic zone, although mean values were greater than control following isoprenaline between 5 and 12.5 minutes of occlusion, they did not differ significantly. In PA FRP was significantly shortened at 2.5 minutes ($P < 0.02$) after isoprenaline

Mean values of RP gradients from the above data are shown in Fig. 40 and Table 25. After isoprenaline the gradient RP CA - PA was significantly greater than control measurements ($P < 0.05$) at 7.5 minutes and also 15 minutes after occlusion ($P < 0.05$). RP NA - PA was significantly increased ($P < 0.05$) at 7.5 minutes. No significant

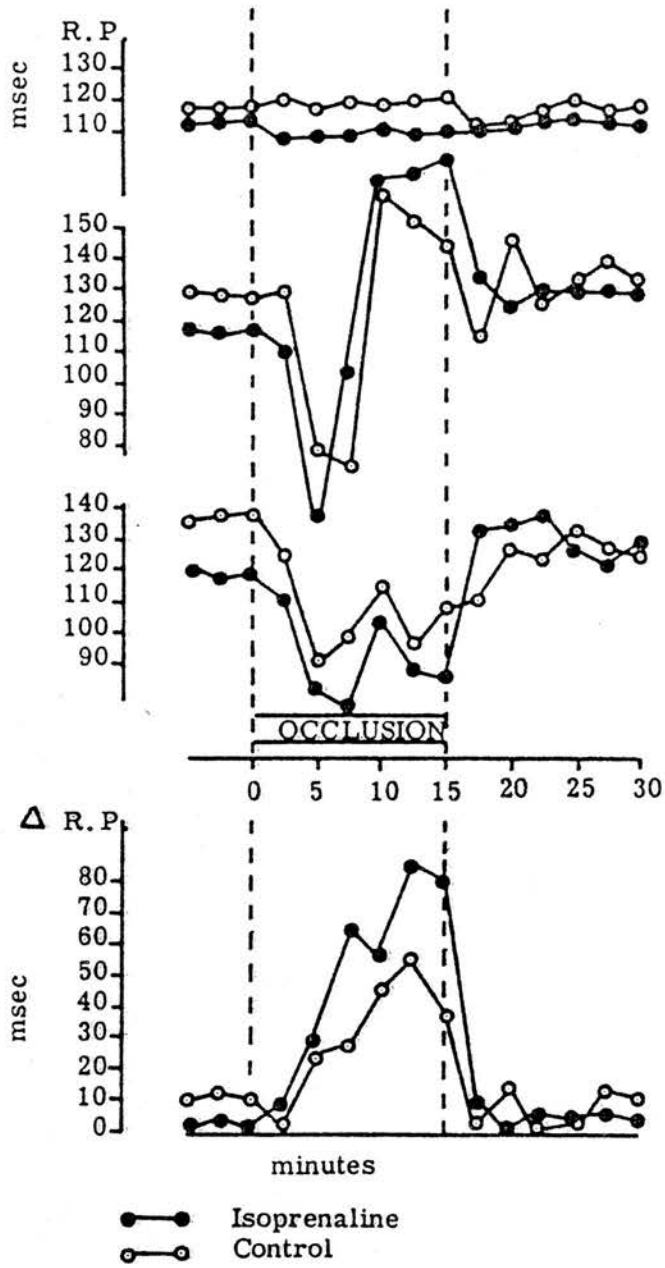


FIG. 38. Changes in refractoriness in normal (NA), central ($IA_1$) and peripheral ($IA_2$) myocardium during a 15 minute occlusion. A control occlusion (open circles) and an occlusion during infusion of isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ (closed circles) are shown. The difference in RP ($\Delta RP$) between the two adjacent ischaemic areas is increased during isoprenaline infusion

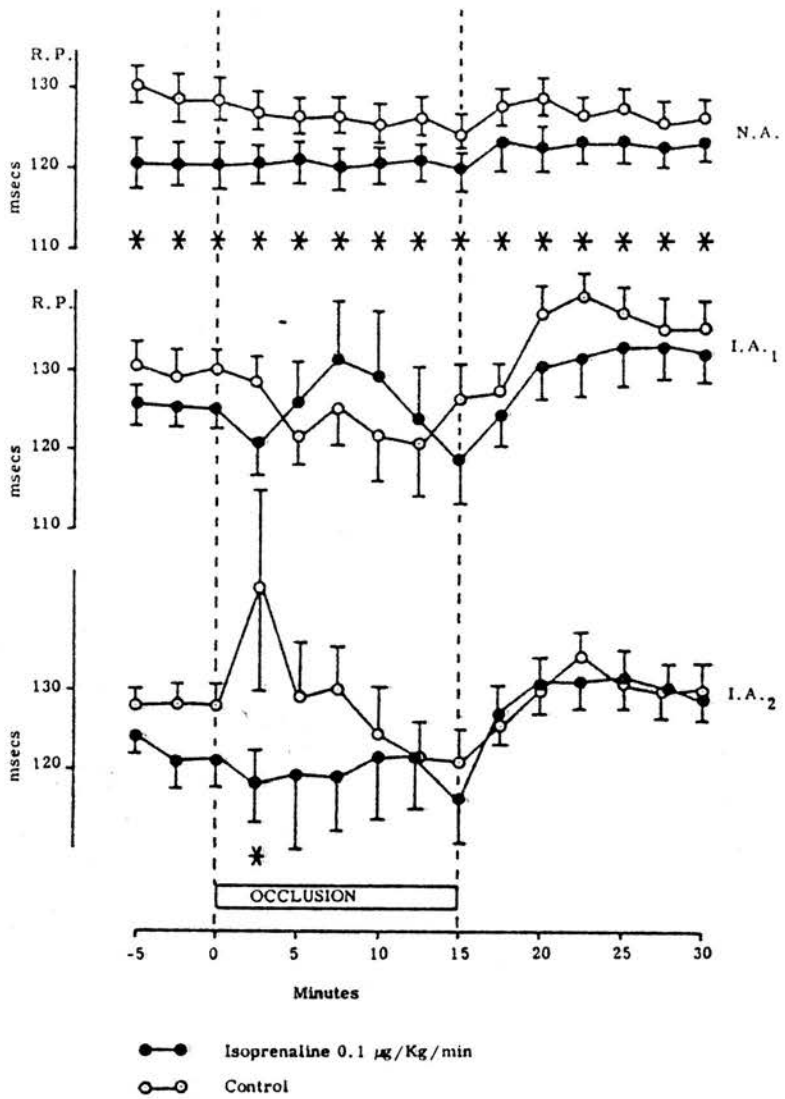


FIG. 39. Mean changes in refractoriness in normal (NA) and adjacent ischaemic ($IA_1$, $IA_2$) myocardium during and after coronary occlusion in 9 dogs. Open circles represent control measurements and closed circles measurements during isoprenaline infusion 0.1 $\mu g.Kg^{-1}.min^{-1}$ at the same heart rate

TABLE 24 Effect of isoprenaline infusion 0.1 $\mu g.Kg^{-1}.min^{-1}$ on changes (mean ± S.E.M.) in refractoriness during and after coronary occlusion in 9 dogs

| | FRP mS | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Isoprenaline | | |
| | NA | CA | PA | NA | CA | PA |
| Minutes after occlusion | | | | | | |
| 0 | 128.4 ± 3.0 | 130.2 ± 3.8 | 127.1 ± 2.9 | 121.5 ± 3.8* | 125.6 ± 3.9 | 122.2 ± 3.2 |
| 2½ | 127.2 ± 2.9 | 127.2 ± 2.9 | 143.0 ± 13.1 | 121.4 ± 3.4* | 121.4 ± 3.4 | 117.7 ± 4.3* |
| 5 | 126.0 ± 2.6 | 122.6 ± 4.7 | 128.2 ± 6.7 | 122.0 ± 3.5* | 126.2 ± 6.8 | 119.0 ± 7.8 |
| 7½ | 126.1 ± 2.5 | 125.6 ± 5.1 | 130.1 ± 5.5 | 126.8 ± 3.2* | 132.1 ± 9.7 | 119.0 ± 10.1 |
| 10 | 125.5 ± 2.7 | 122.3 ± 6.3 | 124.9 ± 5.2 | 122.3 ± 3.4 | 129.7 ± 8.8 | 122.8 ± 9.4 |
| 12½ | 126.0 ± 2.8 | 120.5 ± 7.2 | 122.2 ± 4.6 | 122.0 ± 3.4* | 124.3 ± 7.5 | 121.2 ± 8.7 |
| 15 | 124.7 ± 3.0 | 126.1 ± 7.2 | 121.6 ± 5.5 | 120.5 ± 3.8* | 119.8 ± 6.7 | 116.0 ± 7.0 |
| Minutes after reperfusion | | | | | | |
| 2½ | 127.6 ± 2.8 | 127.7 ± 2.8 | 125.6 ± 4.4 | 124.6 ± 3.3* | 124.6 ± 3.3 | 127.5 ± 2.5 |
| 5 | 128.0 ± 2.6 | 137.7 ± 3.8 | 130.5 ± 4.7 | 123.6 ± 3.1* | 130.7 ± 4.9 | 130.4 ± 4.6 |
| 7½ | 126.7 ± 2.5 | 139.0 ± 3.8 | 134.0 ± 3.9 | 124.1 ± 2.7* | 132.6 ± 5.3 | 131.5 ± 4.7 |
| 10 | 127.4 ± 2.8 | 137.4 ± 4.7 | 132.2 ± 3.9 | 124.2 ± 2.9* | 133.0 ± 5.2 | 131.0 ± 4.3 |
| 12½ | 125.9 ± 3.0 | 135.4 ± 4.1 | 130.2 ± 3.1 | 123.6 ± 2.7* | 133.4 ± 5.0 | 130.5 ± 3.6 |
| 15 | 126.8 ± 2.9 | 135.5 ± 4.2 | 130.0 ± 3.1 | 124.3 ± 3.1* | 133.0 ± 4.8 | 129.7 ± 4.1 |

* $P < 0.02$

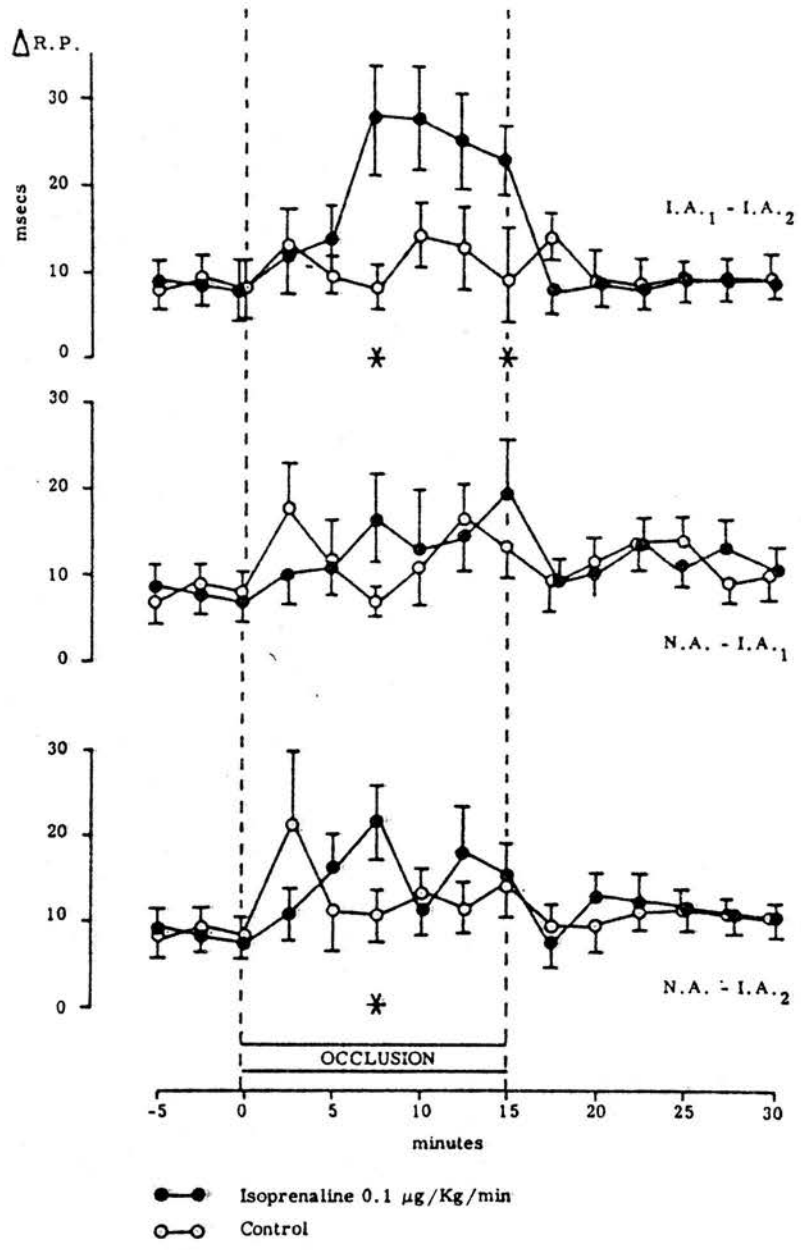


FIG. 40. Mean changes in gradients of refractoriness (ΔRP) between normal and ischaemic myocardium (NA - $IA_1$, NA - $IA_2$) and across ischaemic myocardium ($IA_1$ - $IA_2$) during and after coronary occlusion

TABLE 25 Effect of isoprenaline infusion 0.1 $\mu g.Kg^{-1}.min^{-1}$ on changes (mean ± S.E.M.) of refractory period gradients in the ischaemic zone during and after coronary occlusion in 9 dogs

| | Δ RP mS | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Isoprenaline | | |
| | NA - CA | NA - PA | CA - PA | NA - CA | NA - PA | CA - PA |
| Minutes after occlusion | | | | | | |
| 0 | 8.7 ± 1.8 | 8.4 ± 2.2 | 8.4 ± 2.2 | 6.8 ± 1.6 | 7.9 ± 1.9 | 7.9 ± 1.9 |
| 2½ | 17.8 ± 5.3 | 22.0 ± 10.0 | 13.5 ± 5.2 | 10.0 ± 2.7 | 10.6 ± 3.6 | 13.0 ± 5.5 |
| 5 | 12.7 ± 3.2 | 12.0 ± 4.1 | 9.1 ± 1.9 | 10.2 ± 2.2 | 16.0 ± 4.6 | 14.0 ± 4.8 |
| 7½ | 7.8 ± 1.7 | 11.0 ± 3.8 | 8.7 ± 3.0 | 16.8 ± 6.5 | 22.4 ± 5.9* | 27.6 ± 8.4* |
| 10 | 11.8 ± 4.6 | 12.8 ± 3.9 | 14.5 ± 4.3 | 13.8 ± 7.2 | 10.7 ± 3.0 | 27.5 ± 7.2 |
| 12½ | 16.9 ± 4.2 | 11.3 ± 3.4 | 13.7 ± 5.2 | 14.1 ± 4.7 | 17.2 ± 6.0 | 25.1 ± 6.2 |
| 15 | 13.6 ± 2.6 | 13.6 ± 3.3 | 9.2 ± 5.5 | 18.0 ± 7.7 | 12.4 ± 4.0 | 23.3 ± 4.1 |
| Minutes after reperfusion | | | | | | |
| 2½ | 9.8 ± 2.5 | 9.3 ± 1.2 | 14.2 ± 2.3 | 9.7 ± 1.9 | 7.6 ± 3.0 | 7.9 ± 2.1 |
| 5 | 11.8 ± 2.4 | 9.7 ± 2.9 | 8.7 ± 3.5 | 10.6 ± 2.2 | 13.7 ± 2.7 | 9.4 ± 2.4 |
| 7½ | 13.0 ± 2.6 | 10.5 ± 2.1 | 8.3 ± 3.3 | 13.1 ± 2.9 | 12.4 ± 3.3 | 8.0 ± ].0 |
| 10 | 13.6 ± 2.2 | 11.5 ± 2.0 | 9.9 ± 2.1 | 11.6 ± 2.6 | 11.3 ± 2.8 | 9.0 ± 1.5 |
| 12½ | 8.0 ± 1.7 | 10.5 ± 1.4 | 9.2 ± 2.4 | 13.1 ± 2.8 | 10.2 ± 2.3 | 9.5 ± 1.8 |
| 15 | 10.0 ± 2.4 | 10.1 ± 1.1 | 9.7 ± 2.5 | 10.0 ± 2.4 | 10.0 ± 2.5 | 8.0 ± 2.4 |

*$P < 0.01$

difference was found, however, after isoprenaline for ΔRP NA - CA between normal and control ischaemic zones.

On release FRP followed values of the control run again with a significantly lower value ($P < 0.02$) in the NA and a small reduction in the central area. This difference was not observed in the PA.

Effect of inhibition of lipolysis by nicotinic acid

Mean values of RP before and after inhibition of isoprenaline induced lipolysis are shown in Fig. 41 in eight dogs before, during and after coronary occlusion. Values are given in Table 26.

Although no significant differences were found in RP, NA, CA or PA, results in different dogs were variable. The general trend was of a reduction in the severity of directional change whether this was prolongation or shortening of RP.

An individual example is shown in Fig. 42. The shortening in RP during coronary occlusion was reduced in both CA and PA, with some prolongation of RP in CA in the latter half of the occlusion.

Mean values of RP gradients are shown in Fig. 43 and tabulated in Table 27. In gradients between all three recording areas there was a reduction in mean gradient during coronary occlusion, during nicotinic acid infusion.

Significant levels of difference were not obtained for each individual gradient, but on pooling data from all three gradients a significant reduction in ΔRP was found ($P < 0.01$) after 7½ minutes and 10 minutes of ischaemia. On reperfusion a significant reduction in gradient occurred between 7½ and 15 minutes after release ($P < 0.01$).

Metabolic data

In view of difficulties in local vein sampling in this study, coronary sinus blood sampling was performed. During infusion of

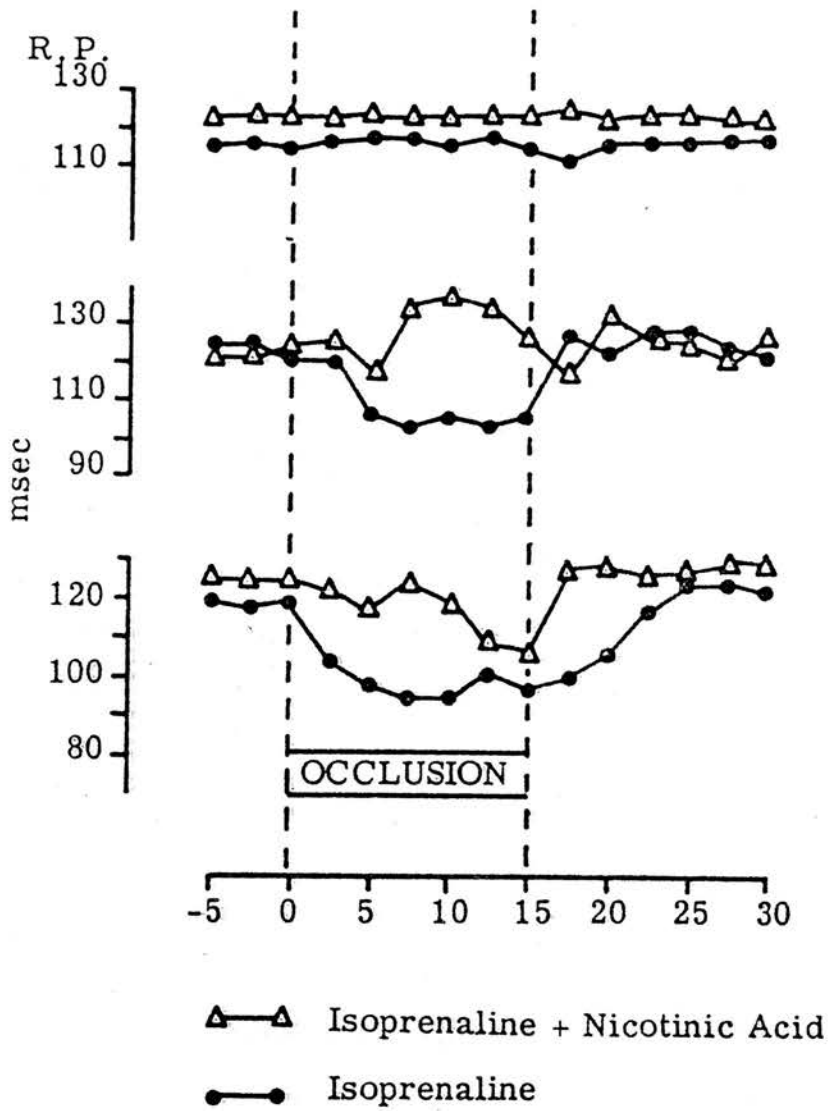


FIG. 41. Example of the effect of inhibition of isoprenaline stimulated lipolysis by nicotinic acid on ventricular refractory periods determined in normal (upper panel), central ischaemic (middle panel) and border ischaemic (lower panel) myocardium following coronary occlusion in one dog. Isoprenaline was infused at 0.1 $\mu g.Kg^{-1}.min^{-1}$ and nicotinic acid 0.5 $mg.Kg^{-1}.min^{-1}$. Heart rate was maintained constant by atrial pacing

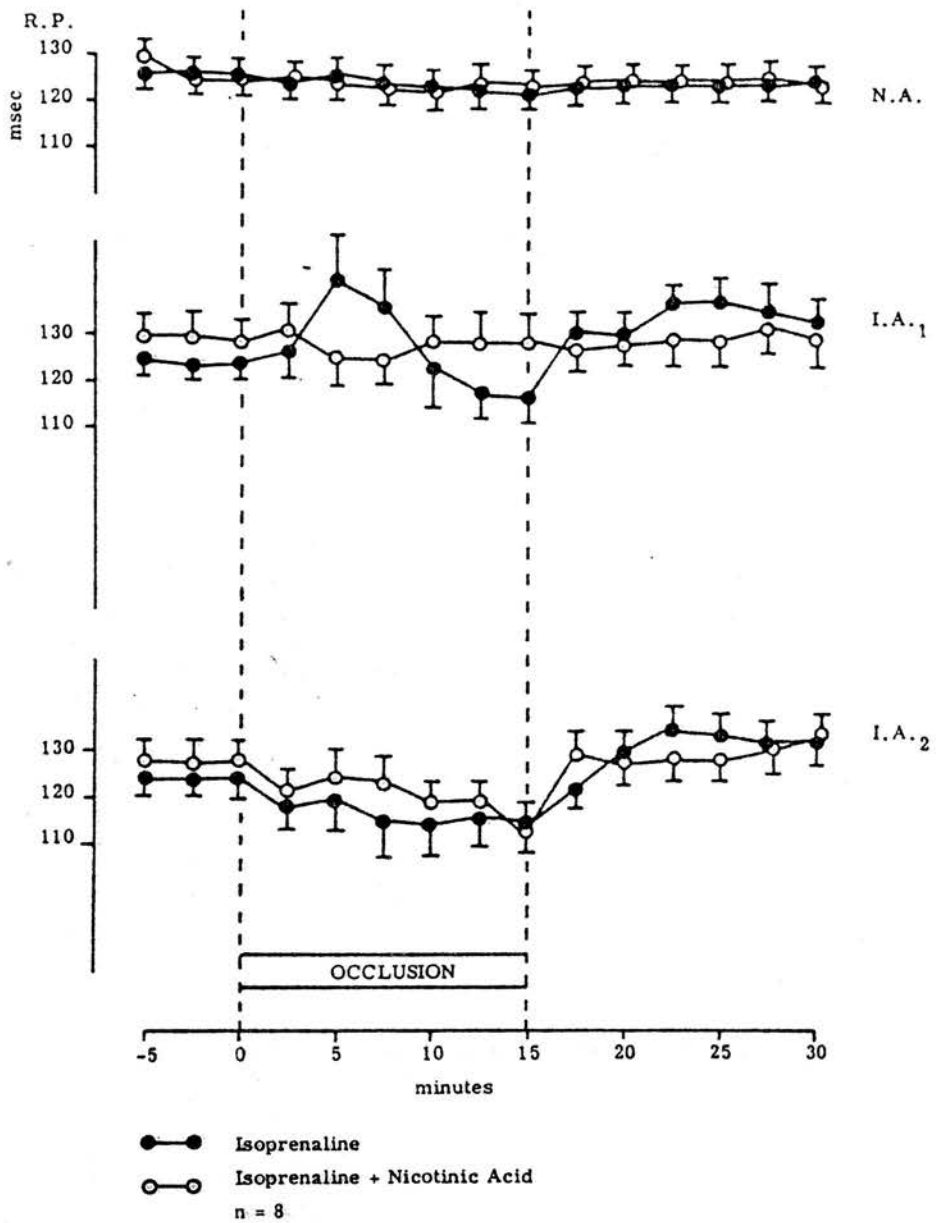


FIG. 42. Mean changes in refractory period in normal (NA) and adjacent ischaemic ($IA_1$, $IA_2$) myocardium during and after coronary occlusion in 8 dogs during infusion of isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ and following inhibition of lipolysis by addition of nicotinic acid 0.5 $mg.Kg^{-1}.min^{-1}$ at constant heart rate

TABLE 26 Effect of inhibition of isoprenaline induced lipolysis by nicotinic acid on ventricular refractoriness during and after coronary occlusion in 8 dogs. Infusion rates of isoprenaline were 0.1 μg.Kg-1.min-1 and nicotinic acid 0.05 mg.Kg-1.min-1

| | FRP mS | | | | | |
|---|---|---|---|---|---|---|
| | Isoprenaline | | | Isoprenaline + Nicotinic Acid | | |
| | NA | CA | PA | NA | CA | PA |
| Minutes after occlusion | | | | | | |
| 0 | 125.1 ± 3.3 | 124.9 ± 4.0 | 124.9 ± 4.0 | 125.5 ± 3.8 | 128.1 ± 4.9 | 127.6 ± 4.5 |
| 2½ | 124.1 ± 3.2 | 126.4 ± 5.9 | 118.4 ± 6.1 | 125.0 ± 4.2 | 131.5 ± 7.6 | 121.4 ± 5.4 |
| 5 | 124.3 ± 3.4 | 141.6 ± 17.9 | 120.0 ± 8.5 | 124.1 ± 3.7 | 125.5 ± 8.6 | 124.5 ± 6.2 |
| 7½ | 124.2 ± 3.7 | 136.2 ± 11.3 | 115.9 ± 9.1 | 123.6 ± 4.6 | 125.5 ± 6.2 | 123.8 ± 6.7 |
| 10 | 123.5 ± 3.7 | 123.6 ± 10.4 | 115.2 ± 8.1 | 122.1 ± 3.9 | 129.0 ± 6.7 | 117.5 ± 4.4 |
| 12½ | 122.9 ± 3.5 | 118.1 ± 7.4 | 116.2 ± 6.5 | 125.0 ± 3.5 | 128.8 ± 7.4 | 117.2 ± 4.1 |
| 15 | 121.1 ± 3.6 | 116.2 ± 7.9 | 115.8 ± 7.2 | 124.1 ± 7.2 | 129.7 ± 7.2 | 112.1 ± 3.0 |
| Minutes after reperfusion | | | | | | |
| 2½ | 123.2 ± 3.5 | 130.1 ± 5.2 | 122.7 ± 5.6 | 124.3 ± 3.4 | 127.1 ± 6.7 | 130.9 ± 5.4 |
| 5 | 123.6 ± 3.2 | 130.0 ± 5.3 | 130.0 ± 5.9 | 124.1 ± 3.5 | 129.4 ± 6.7 | 129.6 ± 6.9 |
| 7½ | 123.8 ± 3.2 | 137.4 ± 5.5 | 135.2 ± 5.1 | 1 24.2 ± 3.9 | 129.6 ± 6.8 | 129.5 ± 5.1 |
| 10 | 123.7 ± 3.0 | 137.5 ± 6.6 | 134.4 ± 4.6 | 124.6 ± 3.9 | 129.2 ± 7.1 | 129.6 + 4.9 |
| 12½ | 124.1 ± 2.8 | 135.1 ± 6.6 | 132.2 ± 3.8 | 125.1 ± 4.8 | 131.2 ± 5.3 | 132.2 ± 5.9 |
| 15 | 124.7 ± 3.1 | 133.5 ± 6.4 | 132.2 ± 4.3 | 123.9 ± 4.6 | 129.4 ± 6.6 | 133.3 ± 5.3 |

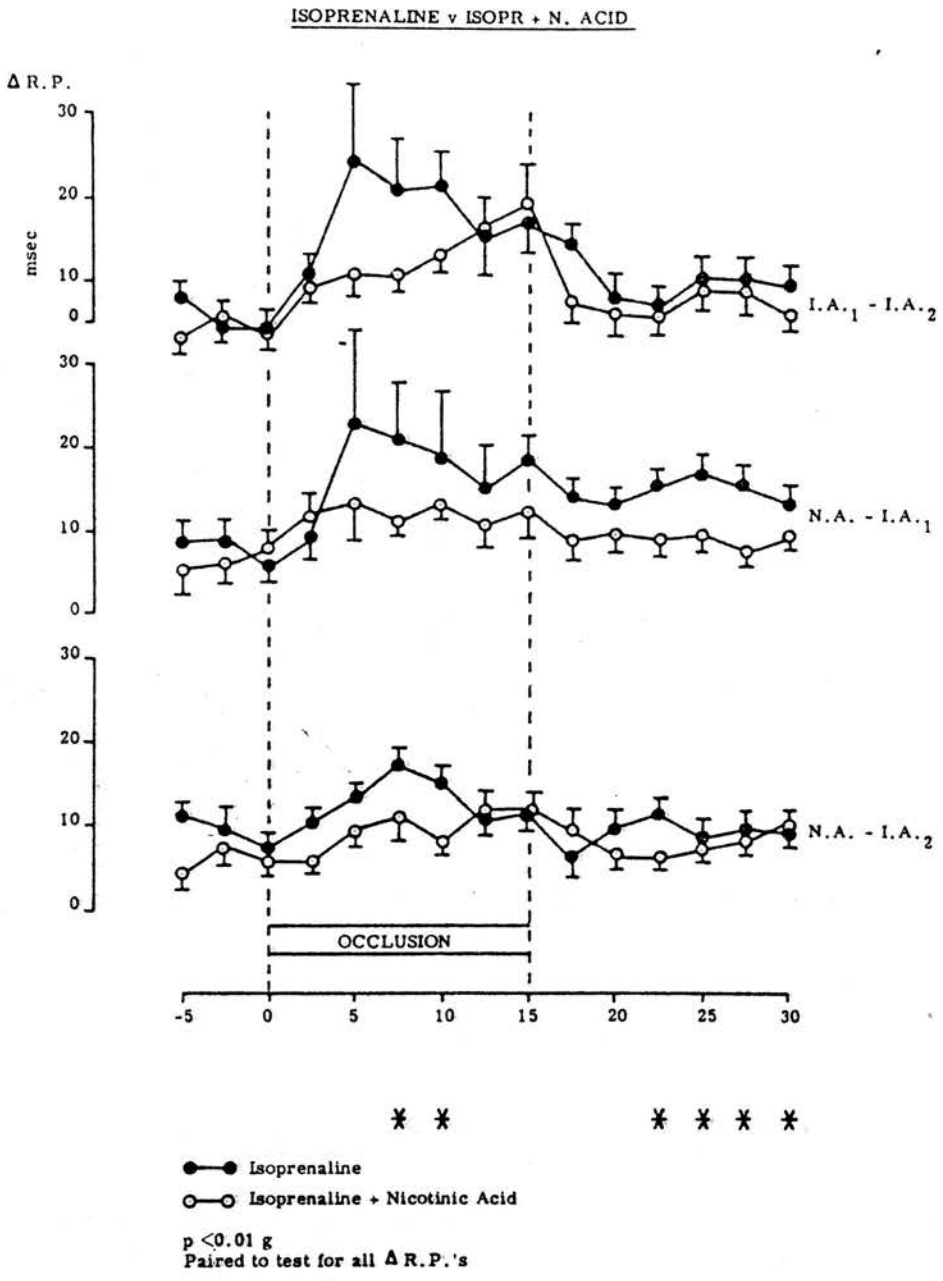


FIG. 43. Effect of inhibition of isoprenaline stimulated lipolysis by nicotinic acid on mean changes in gradients of refractoriness between normal and ischaemic myocardium (NA - $IA_1$, NA - $IA_2$) and across ischaemic myocardium ($IA_1$ - $IA_2$) in 8 dogs. Significant reductions of gradients of refractoriness occurred at 7½ and 10 minutes of ischaemia

TABLE 27 Effect of inhibition of isoprenaline induced lipolysis by nicotinic acid on refractory period gradients during and after coronary occlusion in 8 dogs. Infusion rates of isoprenaline were 0.1 μg.Kg$^{-1}$.min$^{-1}$ and nicotinic acid 0.05 mg.Kg$^{-1}$.min$^{-1}$

| | Δ RP mS | | | | | | |
|---|---|---|---|---|---|---|---|
| | Isoprenaline | | | Isoprenaline + Nicotinic Acid | | | P+ |
| | NA - CA | NA - PA | CA - PA | NA - CA | NA - PA | CA - PA | |
| Minutes after occlusion | | | | | | | |
| 0 | 6.0 ± 1.4 | 7.0 ± 1.8 | 4.0 ± 0.8 | 5.4 ± 2.0 | 8.8 ± 2.7 | 4.7 ± 1.2 | N.S. |
| 2½ | 9.5 ± 3.0 | 10.5 ± 2.2 | 10.5 ± 2.1 | 5.9 ± 1.5 | 12.0 ± 3.8 | 9.1 ± 1.9 | N.S. |
| 5 | 23.2 ± 14.9 | 13.5 ± 1.6 | 24.8 ± 13.7 | 9.6 ± 2.5* | 13.4 ± 4.2 | 11.7 ± 3.7 | N.S. |
| 7½ | 21.5 ± 7.0 | 17.1 ± 2.3 | 21.1 ± 8.7 | 11.3 ± 3.1 | 11.3 ± 1.5 | 11.4 ± 1.5 | <0.01 |
| 10 | 19.2 ± 8.5 | 25.2 ± 3.0 | 21.6 ± 6.9 | 7.3 ± 1.8* | 13.1 ± 1.2 | 13.5 ± 3.7 | <0.01 |
| 12½ | 15.7 ± 5.9 | 10.5 ± 2.4 | 15.2 ± 5.4 | 11.8 ± 2.8 | 10.1 ± 2.1 | 16.7 ± 5.7 | N.S. |
| 15 | 18.9 ± 4.2 | 11.5 ± 2.2 | 17.4 ± 4.0 | 11.0 ± 2.4 | 12.1 ± 2.1 | 19.9 ± 6.4 | N.S. |
| Minutes after reperfusion | | | | | | | |
| 2½ | 14.1 ± 2.6 | 6.5 ± 2.9 | 14.5 ± 2.4 | 9.7 ± 4.1 | 8.8 ± 3.0 | 7.1 ± 2.1 | N.S. |
| 5 | 13.6 ± 2.2 | 9.6 ± 3.1 | 8.5 ± 2.4 | 6.7 ± 1.6 | 9.5 ± 1.9 | 6.9 ± 2.2 | N.S. |
| 7½ | 15.7 ± 2.8 | 11.4 ± 3.0 | 7.9 ± 2.2 | 6.2 ± 1.7 | 9.1 ± 2.0 | 6.6 ± 2.2 | <0.01 |
| 10 | 16.9 ± 3.8 | 8.8 ± 2.4 | 10.6 ± 2.5 | 7.2 ± 2.2 | 9.2 ± 2.1 | 8.7 ± 1.6 | <0.01 |
| 12½ | 15.5 ± 3.7 | 9.5 ± 2.5 | 10.2 ± 2.2 | 7.9 ± 2.9 | 7.5 ± 1.7 | 8.1 ± 2.0 | <0.01 |
| 15 | 13.7 ± 2.9 | 9.0 ± 2.6 | 9.0 ± 2.0 | 10.5 ± 3.2 | 9.4 ± 1.9 | 6.0 ± 1.9 | <0.01 |

*probability refers to differences in grouped Δ RP between isoprenaline and isoprenaline + nicotinic acid and infusion

isoprenaline arterial plasma FFA rose from 393 ± 21 $\mu Eq.l^{-1}$ to 1076 ± 133 $\mu Eq.l^{-1}$ and coronary sinus FAA from 413 ± 35 $\mu Eq.l^{-1}$ to 1038 ± 123 $\mu Eq.l^{-1}$.

After infusion of nicotinic acid arterial FFA fell to 494 ± 42 $\mu Eq.l^{-1}$ ($P < 0.01$) and coronary sinus FFA fell to 452 ± 46 $\mu Eq.l^{-1}$ ($P < 0.01$).

Summary

1. Isoprenaline infusion caused significant shortening of refractory period before and during ischaemia and increased dispersion of refractoriness during ischaemia.

2. Inhibition of lipolysis by nicotinic acid following acute coronary occlusion resulted in:-

   a) significant reductions in arterial plasma FFA concentrations

   b) no significant changes in mean refractory period

   c) significant reductions in mean refractory period gradients at 7½ and 10 minutes after occlusion

   c) reduction in refractory period gradients on reperfusion after 7½ and 15 minutes

## Effect of inhibition of isoprenaline stimulated lipolysis by nicotinic acid on intracellular and extracellular potential recordings

The effect of inhibition of isoprenaline stimulated lipolysis by nicotinic acid was examined following a low coronary occlusion in 8 dogs.

### Electrophysiological effects

Mean data from coronary occlusions during infusion of isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ is contrasted with data from coronary occlusions during combined infusion of isoprenaline 0.1 $\mu g.Kg^{-1}.min^{-1}$ and nicotinic acid 0.5 $mg.Kg^{-1}.min^{-1}$ in Fig. 44 and in Table 28. Changes in APD, endocardial-epicardial CT and ST-segment elevation are shown.

A progressive shortening in APD occurred during isoprenaline treated occlusions from 153 $\pm$ 4 to 126 $\pm$ 7 mS after 5 minutes of occlusion. CT remained unaltered for 2½ minutes of ischaemia, but thereafter showed a mild mean prolongation of 12 mS by 5 minutes.

Small but significant (on paired t-testing) reductions in APD shortening followed antilipolytic therapy, both before ($P < 0.05$) and at 1 minute ($P < 0.001$), 1½ minutes ($P < 0.001$), 2, 2½, 3, 3½ and 4 minutes ($P < 0.05$) of ischaemia. Similarly, a very small but significant ($P < 0.05$) reduction in CT was observed after 4 and 4½ minutes of ischaemia.

Mean ST-segment elevation was significantly reduced throughout the occlusion period.

### Haemodynamic effects

No significant changes in left ventricular dp/dt followed addition of nicotinic acid either before or during coronary occlusion.

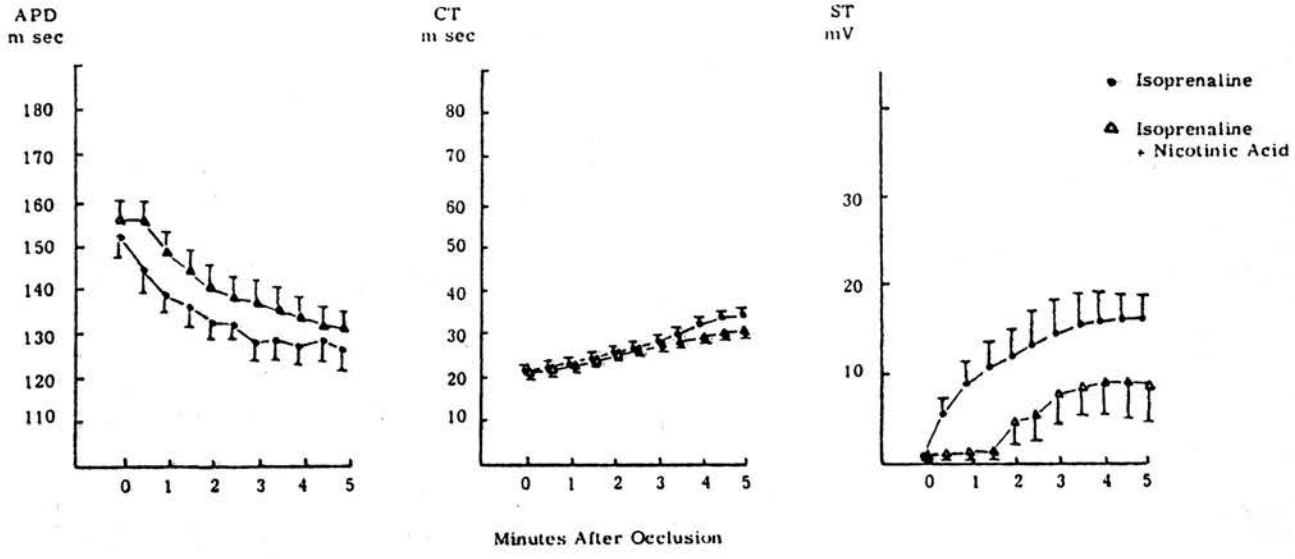


FIG. 44. Effect of inhibition of lipolysis on action potential duration (APD), endocardial-epicardial conduction delay (CT), and epicardial ST-segment elevation following coronary occlusion in 8 dogs. APD shortening and ST-segment elevation were significantly reduced during ischaemia. Slight prolongation of APD preceding occlusion was observed

TABLE 28 The effect of inhibition of isoprenaline stimulated lipolysis by nicotonic acid on action potential duration, endocardial-epicardial conduction time and ST segment elevation in 8 dogs

| Minutes after occlusion | APD mS | | CT mS | | ST mV | |
|---|---|---|---|---|---|---|
| | Isoprenaline | Isoprenaline + Nicotinic Acid | Isoprenaline | Isoprenaline + Nicotinic Acid | Isoprenaline | Isoprenaline + Nicotinic Acid |
| 0 | 153 ± 4 | 158 ± 6* | 22 ± 1 | 23 ± 1 | 0.4 ± 0.3 | |
| ½ | 146 ± 8 | 157 ± 6 | 22 ± 1 | 23 ± 1 | 6.8 ± 2.0 | 1.1 ± 0.7 |
| 1 | 139 ± 7 | 148 ± 7** | 23 ± 1 | 23 ± 1 | 9.6 ± 2.5 | 1.5 ± 1.1 |
| 1½ | 136 ± 6 | 145 ± 7† | 25 ± 1 | 25 ± 1 | 11.2 ± 3.2 | 6.7 ± 1.1 |
| 2 | 134 ± 7 | 141 ± 8* | 26 ± 1 | 26 ± 1 | 12.5 ± 3.1 | 8.6 ± 2.1 |
| 2½ | 131 ± 7 | 139 ± 8* | 27 ± 1 | 27 ± 3 | 13.8 ± 4.5 | 8.9 ± 2.5 |
| 3 | 128 ± 6 | 138 ± 8* | 29 ± 1 | 28 ± 1 | 15.5 ± 4.4 | 9.3 ± 2.5 |
| 3½ | 128 ± 7 | 138 ± 8* | 30 ± 2 | 29 ± 1 | 16.4 ± 4.4 | 9.9 ± 2.6 |
| 4 | 127 ± 7 | 136 ± 8* | 32 ± 2 | 30 ± 2* | 17.1 ± 4.4 | 9.8 ± 2.5 |
| 4½ | 129 ± 7 | 135 ± 8 | 33 ± 2 | 31 ± 2* | 16.8 ± 4.4 | 9.8 ± 2.5 |
| 5 | 126 ± 7 | 133 ± 9 | 34 ± 3 | 32 ± 2 | 16.8 ± 4.4 | 9.8 ± 2.5 |

†P < 0.001

**P < 0.01

*P < 0.05

dp/dt during isoprenaline infusion fell from 4639 ± 620 mm Hg.sec$^{-1}$ to 4077 ± 598 mm Hg.sec$^{-1}$ at 5 minutes of ischaemia. After addition of nicotinic acid, values were 3938 ± 525 and 3691 ± 473 respectively.

Similarly, no significant differences in aortic blood pressure occurred.

Metabolic effects

Arterial levels and metabolic gradients (arterial-local venous differences) of glucose, FFA, glycerol, lactate and potassium are shown in Table 29.

Inhibition of lipolysis is shown by a significant fall in arterial levels of FFA from 1268 ± 151 to 580 ± 54 μE.l$^{-1}$ ($P < 0.001$) and of arterial levels of glycerol from 145 ± 23 to 50 ± 5 ($P < 0.001$).

The arterial-local venous difference of FFA was reduced both before and during ischaemia by nicotinic acid, but not significantly so.

Arterial glucose levels were slightly increased during isoprenaline infusion to 6.36 ± 0.39 mM, but were not significantly altered by inhibition of lipolysis. Arterial local venous differences of glucose were increased during ischaemia ($P < 0.05$), this increase being slightly greater, although not significantly greater during inhibition of lipolysis.

Arterial lactate levels were slightly elevated throughout the study. Arterial-local venous differences of lactate were less negative during ischaemia after nicotinic acid, but not significantly so.

Arterial potassium levels and the increased arterial-local venous differences of potassium during ischaemia were not influenced by inhibition of lipolysis.

TABLE 29 Effect of nicotinic acid induced inhibition of lipolysis on metabolic gradients across ischaemic myocardium during electrophysiological studies of low coronary occlusion in 8 dogs

| | Minutes after occlusion | | | |
|---|---|---|---|---|
| | Isoprenaline | | Isoprenaline + Nicotinic Acid | |
| | 0 | 5 | 0 | 5 |
| otassium μM | | | | |
| Arterial | 3.4 ± 0.20 | 3.3 ± 0.19 | 3.2 ± 0.24 | 3.2 ± 0.16 |
| Local venous | 3.8 ± 0.24 | 5.2 ± 0.73 | 3.4 ± 0.37 | 4.75 ± 0.39 |
| A-1v | -0.5 ± 0.18 | -1.6 ± 0.71 | -0.5 ± 0.19 | -1.5 ± 0.20 |
| actate mM | | | | |
| Arterial | 2.2 ± 0.35 | 1.85 ± 0.84 | 2.54 ± 0.9 | 1.84 ± 0.52 |
| Local venous | 2.55 ± 0.34 | 5.41 ± 1.6 | 2.56 ± 0.91 | 5.18 ± 0.89 |
| A-1v | -0.43 ± 0.21 | -3.35 ± 0.60 | -0.47 ± 0.15 | -2.08 ± 1.31 |
| ree fatty acid μM | | | | |
| Arterial | 1268 ± 151 | 1142 ± 126 | 580 ± 54+ | 660 ± 56+ |
| Local venous | 1205 ± 142 | 1046 ± 126 | 533 ± 40+ | 571 ± 95+ |
| A-1v | 125 ± 67 | 242 ± 89 | -64 ± 61 | 12.5 ± 52 |
| lycerol | | | | |
| Arterial | 144.8 ± 22.6 | 152.5 ± 25.6 | 49.8 ± 4.8+ | 66.0 ± 7.8+ |
| Local venous | 145.0 ± 14.0 | Ins | 44.0 ± 4.5+ | Ins |
| A-1v | -2.8 ± 13.8 | Ins | -14.8 ± 5.9 | Ins |
| ucose mM | | | | |
| Arterial | 6.36 ± 0.30 | 6.08 ± 0.40 | 6.43 ± 0.41 | 6.31 ± 0.43 |
| Local venous | 6.03 ± 0.38 | 4.63 ± 0.20 | 6.08 ± 0.33 | 5.28 ± 0.30 |
| A-1v | 0.36 ± 0.16 | 0.72 ± 0.20 | 0.35 ± 0.18 | 1.01 ± 0.28 |

Regional myocardial blood flow

No significant changes in endocardial or epicardial regional myocardial blood flow were induced by addition of nicotinic acid to the infusion of isoprenaline prior to coronary occlusion (Table 30). Three and a half minutes after coronary occlusion endocardial and epicardial regional myocardial blood flow were reduced to 39% and 53% respectively of pre-occlusion values in the ischaemic zone during isoprenaline infusion. Following inhibition of isoprenaline stimulated lipolysis the reductions in flow in the ischaemic region after coronary occlusion were 38% and 43% in endocardium and epicardium respectively. Absolute values of flow did not differ significantly.

BLE 30 Effect of inhibition of isoprenaline stimulated lipolysis by nicotinic acid on regional myocardial blood flow determined before and 3½ minutes after coronary occlusion during electrophysiological studies in 5 dogs

| | Regional Myocardial Blood Flow $ml.g^{-1}.min^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Isoprenaline | | | Isoprenaline + Nicotinic Acid | | |
| | Endo | Epi | Endo/Epi | Endo | Epi | Endo/Epi |
| Before occlusion | | | | | | |
| Normal Zone | 0.94 ± 0.09 | 0.94 ± 0.08 | 1.03 ± 0.05 | 0.95 ± 0.08 | 1.01 ± 0.06 | 0.94 ± 0.05 |
| Ischaemic Zone | 0.71 ± 0.10 | 0.77 ± 0.09 | 0.94 ± 0.08 | 0.73 ± 0.05 | 0.89 ± 0.03 | 0.85 ± 0.05 |
| During occlusion | | | | | | |
| Normal Zone | 0.95 ± 0.23 | 1.16 ± 0.18 | 0.89 ± 0.13 | 0.93 ± 0.22 | 1.16 ± 0.21 | 0.96 ± 0.15 |
| Ischaemic Zone | 0.28 ± 0.07 | 0.41 ± 0.08 | 0.58 ± 0.10 | 0.28 ± 0.07 | 0.39 ± 0.04 | 0.72 ± 0.20 |

Summary

Inhibition of isoprenaline induced lipolysis by nicotinic acid during low coronary occlusion induced:-

1. Small, but significant, reduction in APD shortening between 1 and 4 minutes of ischaemia.
2. Slight prolongation of APD before occlusion.
3. Small, but significant, reduction in CT at 4 and 4½ minutes of ischaemia.
4. Reduction in arterial plasma FFA and arterial plasma glycerol levels.
5. Reduction in arterial-local venous gradient of FFA.
6. No change in lactate or potassium metabolic gradients.
7. No detectable haemodynamic effects.
8. No effect on regional myocardial blood flow in endocardium or epicardium in normal or ischaemic zones of myocardium.

# DISCUSSION

# DISCUSSION

## 12. INTRODUCTION

Early out-of-hospital ventricular fibrillation is believed responsible, in large part, for the enormous death toll in man from coronary heart disease and has been related to the acute phase of enhanced ventricular vulnerability to arrhythmias within the first few minutes following experimental coronary occlusion in the dog (Bigger, 1977). Common pathogenetic mechanisms may link the high incidence of arrhythmic deaths within the first 15 minutes of onset of symptoms of myocardial ischaemia in man (Gordon and Kannel, 1971; Oliver, 1976; Kuller et al, 1975) with this early arrhythmic phase (Harris, 1950) which occurs between three and six minutes of coronary occlusion in the dog, together with the marked dip at this time in ventricular fibrillation threshold (Wiggers et al, 1940; Han, 1969; Burgess et al, 1971) and in ventricular premature beat threshold in this study. Although little data is available from man over this period, studies of the pre-hospital phase of infarction (Adgey et al, 1971) or of acute changes following the presumed coronary arterial spasm of Prinzmetal variant angina (Kleinfeld and Rozanski, 1976), suggest that similar electrophysiological changes occur in man as in the dog, which may precipitate arrhythmogenesis.

The experimental parallel is by no means complete. The degree of ischaemia following occlusion relates to the extent of coronary collateralisation which can be considerably more extensive in the dog than in man (Schaper, 1971). Anaesthesia suppresses the general systemic response to ischaemia and inhibits reflex effects of probable importance in the genesis of arrhythmias (Oliver, 1972).

Plasma free fatty acid levels were depressed in all studies and plasma catecholamines would have been reduced likewise. Destruction of fine sympathetic afferent and efferent nerve fibres is unavoidable on dissection of the left anterior descending coronary artery and may have effected a partial or complete regional sympathectomy. Both afferent reflex discharge (Brown, 1967; Malliani et al, 1969) and tonic or phasic efferent sympathetic tone could be diminished. The antiarrhythmic effect of sympathectomy is well known (Kliks et al, 1972) and neurogenic effects mediated by the sympathetic system are of major importance in the genesis of early arrhythmias (Lown and Verrier, 1976; Schwartz et al, 1976). Closer experimental control may have been achieved, however, by at least partial removal of this uncontrolled variable.

In addition, the electrophysiological response is determined by the site of coronary occlusion. Fibrillation is more likely to be precipitated from a large ischaemic zone and proximal occlusion (Bloor et al, 1975). The response to circumflex arterial or septal arterial occlusion may differ both in terms of arrhythmogenic and reflex response from occlusion of the anterior descending coronary artery (Oliver, 1972).

Finally, the overall haemodynamic response to ischaemia may be influenced by physiological consequences of thoracotomy and the open chest preparation by reduction of venous return, alteration of atrial filling pressure and possible local cooling effects on the myocardium.

Any attempt at drawing parallels between effects in the experimental model adopted of acute coronary occlusion in the dog and effects in conscious man must be viewed in the light of these provisos.

## 13. ELECTROPHYSIOLOGY OF ISCHAEMIA

Complex electrophysiological changes occur within the first few minutes of myocardial ischaemia, whose precise aetiology remain largely undefined. The consistent finding of a nadir in VPB threshold curve between three and seven minutes after coronary occlusion corresponding exactly with the period of emergence of spontaneous ventricular premature beats confirms the emergence of a phase of enhanced vulnerability to induction of arrhythmias (Harris, 1950; Burgess et al, 1971). Values reverted to preocclusion levels after 15 minutes. Battle et al (1974) performed repeated coronary occlusions of varying duration and similarly found a significant decrease in VFT after three to six minutes of ischaemia and no significant change after 15 to 45 minutes. The degree of fall in ventricular fibrillation threshold is greater the less the coronary collateral circulation to the ischaemic zone (Garza et al, 1974; Messman et al, 1976) and can be partially reversed by β-blockade (Schley et al, 1973). The degree of enhancement of vulnerability thus can be related to both severity of ischaemia and sympathetic or catecholamine activity. A further important determinant of such vulnerability is inhomogeneity of perfusion (Han and Moe, 1964). Isoprenaline infusion induced a transient fall in fibrillation threshold followed by an elevation above normal in normal myocardium. By contrast, sympathetic trunk (stellate ganglia) stimulation induced a sustained fall of threshold (Han et al, 1964). Han suggests therefore that transient initial inhomogeneities of perfusion of isoprenaline in the myocardium may account for transient enhancement of vulnerability, whereas nerve stimulation might produce local heterogenity of response according to the location of sympathetic nerve terminals in the myocardium.

The reduction in VPB thresholds in this study prior to occlusion during infusion of isoprenaline may relate to perfusion inhomogeneity. More prolonged infusion, however, may have abolished the effect.

Several alternative explanations can account for the transient nature of this phase of enhanced vulnerability during acute ischaemia. Heterogeneity of cellular perfusion and electrophysiological response could be maximal at this time. Histological studies of acutely infarcted hearts clearly show inhomogeneity of distribution of cell death within the ischaemic zone (Jennings and Ganote, 1972, 1976). Fluorescence photography from the beating rat heart of the redox or NADH/NAD status of the myocardium during ischaemia (Barlow and Chance, 1976) demonstrates that the inhomogeneity of metabolic function is at first patchy, but then progressively increases until the zones of reduced NADH become confluent. Similar studies are not yet reported in the dog or man. It is likely, however, that ischaemia results initially in a heterogeneous population of both severely ischaemic and relatively well oxygenated cells. Progressive confluence of severely ischaemic zones might then induce a homogeneous zone once more, but of severely depressed tissue. Certainly, local inhomogeneity of refractoriness has been demonstrated in ischaemic tissue (Han, 1969) and been related to reduction of fibrillation threshold (Han, 1964). Inhomogeneity of conduction velocity and variable conduction blocks are prerequisites for re-entrant excitation and arrhythmogenesis (Wit et al, 1974). The early nadir of the ischaemic VPF threshold curve thus could represent a period of metabolic and electrophysiological heterogeneity and the later rise and normalisation of VPB threshold a period of more homogeneous and confluent ischaemia.

An alternative or additive possibility is modulation of the electrophysiological response by reflex sympathetic neural activity, enhanced uptake of circulating adrenaline or massive local phasic release of noradrenaline from sympathetic nerve terminals in the ischaemic myocardium. Direct recording of afferent sympathetic neural activity following coronary occlusion has demonstrated increased traffic in the first few minutes after experimental coronary occlusion (Malliani, 1969).

Noradrenaline release from ischaemic myocardium is enhanced in coronary venous effluent in the dog following occlusion (Shahab et al, 1967, 1972), although incremental release is small compared with that obtained on coronary reperfusion. Elevation of plasma adrenaline occurs rapidly following experimental coronary occlusion in the dog (Staszewska-Barczak and Ceremuzynski, 1968), probably reflexly as the effect is abolished by adrenalectomy (Ceremuzynski et al, 1969). An elevation in plasma adrenaline concentrations is known to increase myocardial adrenaline uptake (Christenson, 1978) which is likely to be inhomogeneously distributed in the ischaemic zone. The fall in VPB threshold found during ischaemia was enhanced dramatically by infusion of isoprenaline, unrelated to alteration of heart rate. Both an enhancement of ischaemia by a catechol induced increase in inotropic state and hence myocardial oxygen consumption (Braunwald, 1971; Maroko et al, 1971) and a direct electrophysiological effect of isoprenaline (Noble, 1975) must be considered as possible mechanisms of action.

Similarly, secondary electrophysiological effects resulting from inhomogeneity of metabolite release, e.g. hydrogen ion, potassium, lactate, adenosine, inosine, etc, together with their

effects on adjacent normally oxygenated cells could influence vulnerability. Following the description of the triphasic pattern of arrhythmias following coronary occlusion (Harris, 1950) potassium release was shown to correlate with the first two phases of arrhythmias (Harris et al, 1954). The initial arrhythmic phase was accompanied by potassium release, whereas the following quiescent phase was not. This association has been confirmed (Regan et al, 1967; Thomas et al, 1976), but is not quantitative. Certainly, intracoronary injection of potassium chloride (Harris et al, 1954) can induce ventricular fibrillation. The arterial local venous difference of potassium was consistently greater after five minutes of ischaemia than after 10 minutes during control VPB threshold studies. This may relate to an initial transient surge of potassium leakage or alternatively be artefactual as a result of regional flow redistribution consequent upon a release of vaso-active metabolites. A transient elevation of extracellular potassium at the time of maximum vulnerability to arrhythmogenesis is, however, possible. An increase in extracellular potassium sufficient to depolarise the fibre to levels less negative than -50 mV results in slowly conducting slow response activity (Cranefield et al, 1972). Transient inhomogenous slow response activity could be a powerful determinant of vulnerability.

A further important determinant of ventricular vulnerability is believed to be inhomogeneity of refractoriness (Han, 1969; Surawicz, 1971; Wit et al, 1974; Lazzara et al, 1978). Local dispersion of ventricular refractoriness within a 4 mm radius of the site of stimulation is increased by sympathetic stimulation, toxic doses of digitalis, chloroform, quinidine and hypothermia

(Han and Moe, 1964; Han et al, 1966) as well as during ischaemia. Slowing of heart rate increases dispersion of refractoriness in addition to lowering ventricular fibrillation threshold (Han et al, 1966; Kent et al, 1973). Studies of changes in ventricular refractoriness during acute ischaemia have been contradictory describing either shortening (Levites et al, 1975), shortening followed by prolongation (Han, 1972; Elharrar et al, 1977) or prolongation alone (Szekeres and Papp, 1971) of the refractory period.

This study demonstrates that a variety of patterns of change of refractoriness occur during acute ischaemia and that different patterns may co-exist in central and border areas of ischaemia. The dominant pattern in the border zone was of a shortening in refractoriness, sometimes with an initial transient prolongation; in the central area either progressive shortening or shortening followed by prolongation of refractoriness were dominant. It has been suggested (Tsuchida, 1965) that shortening of refractoriness may relate to early or mild ischaemia and prolongation to later or severe ischaemia. That both patterns may co-exist simultaneously in the ischaemic region is suggested by studies of diastolic excitability threshold (Elharrar et al, 1977) showing a greater elevation of threshold in the central, compared with border zones. Furthermore, these effects were reproducible following local elevation of extracellular potassium concentrations. As a result of regional divergence in refractoriness, differences or gradients of refractoriness are created. The likelihood of re-entry from an area of longer refractoriness into an area of shorter refractoriness is thus enhanced. Even minute gradients of refractoriness may be of importance. Allesie et al (1976) have demonstrated that

disparities of refractoriness of as little as 11 mS in adjacent areas are sufficient to generate local conduction block and delayed conduction of the type associated with re-entrant activity following application of an extrastimulus in atrial tissue. This study demonstrates an enhancement of gradients of refractoriness between widely separated areas of myocardium associated with early arrhythmogenesis. In particular, ventricular fibrillation was preceded by marked regional divergence in refractoriness over a period of less than five minutes. In some instances gradients of up to 110 mS were recorded. In general, rapid shortening was observed in the more peripheral zone and prolongation in the central and severely ischaemic zone. Gross conduction delays were not recorded in many of these animals, although insensitivity of the local bipolar electrogram may not have revealed some local delays. Changes in refractory gradients per se may then be an important predeterminant of fibrillation. Focal cooling of the epicardium results in more marked prolongation of refractoriness than conduction delay with respect to normal adjacent tissue and is associated with ventricular tachycardia or fibrillation (Wallace and Mignane, 1966). Divergence of refractoriness itself, however, can retard local conduction. If depolarisation is initiated before complete repolarisation of the preceding impulse, propagation will be slowed according to the level of membrane potential from which the action potential arises and to its upstroke velocity. In a region showing marked divergence of refractoriness a re-entrant impulse will be either blocked, slowed or conducted normally so creating a fractionated waveform (Moe and Abildskov, 1959). A similar mechanism has been demonstrated for re-entry as a result of unidirectional conduction block of the Purkinje fibre - ventricular

muscle junction (Sasyniuk and Mendez, 1973). At the point of block regions of depolarised Purkinje fibres and repolarised ventricular muscle fibres are in juxtaposition. The passage of a second impulse or re-entrant impulse can dramatically increase divergence of refractoriness and hence further re-entry in an area demonstrating even minor divergence of refractoriness. In addition, ventricular vulnerability or fibrillation threshold is greatly reduced after an ectopic beat (Han et al, 1966). Refractoriness of premature beats preceding multiple responses and ventricular fibrillation is significantly shorter than in single premature responses (Surawicz et al, 1967). The differences in refractoriness demonstrated by widely separated areas of ischaemic myocardium could therefore initiate a macro-re-entry circuit throughout the whole area which might later fragment into the disorganised electrical activity of ventricular fibrillation.

It is of interest that changes in refractoriness were accelerated and enhanced during isoprenaline infusion and that gradients of refractoriness were increased. The arrhythmogenic effects of catecholamines are well known (Moore et al, 1964; Ceremuzynski et al, 1969), as indeed are their effects in increasing local dispersion of refractoriness (Han et al, 1964). The normal refractory period was shortened in keeping with the known effects of sympathetic stimulation (Tsien et al, 1972). During ischaemia maximal gradients of refractoriness occurred after five minutes of ischaemia at a time when ventricular vulnerability is at a maximum (Lown and Verrier, 1976). Rate-dependent effects were eliminated by overdrive pacing. In vivo, however, catecholamine induced rate-dependent changes may in themselves influence refractoriness. Although a slowing of heart rate per se increases dispersion of refractoriness and decreases fibrillation

threshold (Han, 1969), during ischaemia an increased rate augments the degree of ischaemia with similar effect (Kent et al, 1973). The contribution of changes in refractoriness to catecholamine-induced arrhythmias must, however, be weighed against their effects on enhancement of ventricular automatic activity and induction of slowly conducting 'slow potentials' (Cranefield and Hoffman, 1971).

Mechanisms underlying these alterations in refractoriness during ischaemia are complex. Refractoriness itself, as assessed by application of an extra stimuli at varying coupling intervals, can be defined either in terms of initiation of a propagated response or in terms of the nature of the propagated response (Lazzara et al, 1978) which may be greatly depressed. There appear to be opposing effects of ischaemia on refractoriness. On the one hand action potential shortening might result in a shortened refractory period (Hoffman and Cranefield, 1960). On the other hand a superimposition of post-repolarisation refractoriness (Lazzara et al, 1975) might prolong refractoriness despite shortening of the cardiac action potential (Downar et al, 1977). In the absence of any alteration in diastolic excitability threshold action potential duration and refractoriness should follow pari passu. An elevated threshold as may follow a delayed recovery of the fast inward sodium channel (Gettes and Reuter, 1974) would prolong refractoriness despite shortening of the action potential. Both phenomena would appear operative during ischaemia. Post-repolarisation refractoriness is enhanced with increasing severity of ischaemia and is increased at rapid heart rates (Lazzara et al, 1975).

The phenomenon of reperfusion fibrillation is well described experimentally (Tennant and Wiggers, 1935; Sewell et al, 1955; Battle et al, 1974) and has been related to the sudden washout of

metabolites such as potassium or lactate (Lang et al, 1974). The finding of a greater disparity in refractoriness between central and border ischaemic zones would suggest a further correlate with pre-existing electrophysiological abnormality. There is evidence, however, that re-entrant mechanisms may not be involved in the genesis of this reperfusion fibrillation (Corr et al, 1977). The normal idioventricular escape rate following cervical vagal stimulation in the cat was increased during coronary reperfusion, suggesting an enhancement of automaticity. In addition, abnormal conduction delays in the ischaemic zone, although present during ischaemia, were absent at the time of reperfusion arrhythmia. Certainly, a dip in the VPB threshold curve is demonstrable on reperfusion. It is of interest that it occurs at a time of maximal noradrenaline release from the ischaemic zone (Shahab et al, 1972). It is possible, therefore, that the observed increases in divergence of refractoriness preceding reperfusion fibrillation merely reflect the severity of ischaemia which in turn may relate to the degree of noradrenaline release or other stimulus to automatic activity. No increase in ventricular automaticity in dogs with complete heart block has been shown, however, on coronary perfusion (Levites et al, 1975) and minimal changes were found on overdrive suppression. These authors conclude that there is no enhancement of automaticity at this time. Rapid recovery of refractoriness was observed to follow reperfusion in this study. Large transient gradients of refractoriness could arise, therefore, as a result of asynchronous recovery which could initiate re-entrant activity.

A further prerequisite of re-entry is slowing of conduction.

The studies of combined intracellular and extracellular recordings following high coronary occlusion clearly demonstrate marked slowing of endocardial-epicardial conduction in the central ischaemic zone following coronary occlusion. In animals showing milder ischaemia conduction delay was unaltered over the first one or two minutes of ischaemia, but thereafter was progressively increased. In animals showing severe ischaemia a progressive increase in conduction delay was observed from around 30 seconds after occlusion. Ventricular fibrillation in all cases occurred at a time of maximum ventricular conduction delay. Similar conduction delays in ventricular muscle have been demonstrated by extracellular recording techniques (Durrer et al, 1971; Boineau and Cox, 1973; Williams et al, 1974; Scherlag et al, 1974) following left anterior descending coronary occlusion or anterior septal artery occlusion (El-Sherif et al, 1975) in the dog. Bipolar extracellular recording can, however, only demonstrate electrical activity between two closely applied electrodes over the region of a large number of cells. Similarly, local depolarisation is given by the intrinsic deflection of the unipolar electrogram (Durrer et al, 1953) which may become distorted during severe ischaemia. Fragmentation of the bipolar electrogram occurs during ischaemia (El-Sherif, 1975) suggesting a fragmentation of ventricular activation delays in the cells between the local electrodes. Delays in septal activation of up to 335 mS are reported within five minutes of ischaemia (El-Sherif, 1975). The greatest delay observed in this study was 210 mS, this also being well beyond the time of normal ventricular muscle repolarisation. Distortion of action potential morphology and appearance of additional humps or notches on the action potential were commonly observed. In

view of known electrotonic interaction between adjacent groups of cells in vivo it is likely that these changes represent fragmentation of depolarisation delay in adjacent cells. In some instances such action potential notches were as much as 100 mS separated suggesting considerable local dispersion of activation. The ultimate proof of re-entry would be the demonstration of continuous 'bridging' activity or continuous ventricular depolarisation between either one beat and a re-entrant extrasystole or between re-entrant premature beats. This has not yet been demonstrated during acute ischaemia. Using a large composite bipolar electrode to cover the whole of an ischaemic area in the dog left ventricle El-Sherif et al (1976) have demonstrated such continuous activity three to seven days following coronary ligation. Late 'bridging' diastolic depolarisation preceding arrhythmogenesis was not observed in this study. Intracellular potential recordings were made at only one site which may not have coincided with a site of late diastolic re-entry. Intracellular recordings from the perfused pig heart epicardial surface (Downar et al, 1977; Janse and Downar, 1977) during regional ischaemia show similar large conduction delays. Absolute changes were perhaps slightly lower than those of this study. Important anatomical differences, however, exist between the pig and the dog or man with respect to the distribution of the ventricular specialised conduction tissue (Hamlin et al, 1975; Holland and Brooks, 1976). In the pig the Purkinje cells are distributed throughout the ventricular wall and ramify into the subepicardium. In man and in the dog the Purkinje network is purely subendocardial. Activation in the pig of endocardium and epicardium can occur almost

simultaneously (Hamlin et al, 1975), whereas in the dog it must spread from endocardium to epicardium (Durrer et al, 1964).

Information regarding the possible mechanism of delayed conduction during ischaemia can be obtained from the analysis of changes in action potential morphology during ischaemia. The early acute changes observed in the central ischaemic zone were of an initial action potential shortening, a loss of action potential amplitude and diminution of upstroke velocity (assessed qualitatively) to be followed by a loss of the notch or phase 1 of the action potential preceding the plateau phase and the appearance of potentials of "slow response" type morphology (Cranefield et al, 1972). Initial loss of action potential amplitude has been shown to relate to loss of resting membrane potential and TQ-segment depression (Prinzmetal et al, 1962; Samson and Scher, 1960; Kleber et al, 1978) and should also account for diminution in upstroke velocity and hence conduction velocity of the impulse. Depressed upstroke velocities can occur in isolated Purkinje tissue even at normal resting membrane potentials (Scherlag, 1978). It is possible, therefore, that in ventricular myocardial tissue, which has a slower conduction velocity and hence a lower safety factor than Purkinje tissue, marked slowing of conduction could arise from depression without complete inactivation of the rapid sodium channel responsible for the initial fast upstroke of the action potential (Lazzara et al, 1975). Certainly, factors, as yet unknown, in ischaemia inhibit reactivation of the sodium channel (Carmeliet, 1978). Even in the absence of such effects, however, membrane depolarisation around -60 mV leads to inactivation of the sodium channel and generation of "slow response" potentials of slow upstroke velocity and conduction characteristics (see Introduction).

It has been widely postulated that the genesis of "slow response" potentials must be a key factor in the initiation of the slowed conduction necessary for re-entry (Cranefield, 1975; Wit et al, 1974; Carmeliet, 1978). Action potentials of "slow response" morphology were recorded in this study at a time of significant conduction delay. Such potentials did not occur in the absence of conduction abnormalities. In the absence of absolute transmembrane potential measurements it was not possible to distinguish between a true "slow response" potential and a depressed "fast response" potential. What appear to be true "slow response" potentials do undoubtedly occur during ischaemia and have been found in in vitro studies in areas of myocardial infarction produced by ligation of the anterior descending coronary artery in the dog (Friedman et al, 1973), and in Purkinje fibres from the hearts of old dogs subject to extrasystoles (Cranefield et al, 1973). Potentials of similar morphology were found in the pig ischaemic study of Downar (1977), but are subject to the same difficulties of interpretation. The 'milieu' during acute ischaemia should be expected to provoke slow response activity as in the in vitro preparation. High extracellular potassium levels, known to induce membrane depolarisation and local catecholamine release, which is a slow channel stimulant, are to be found. Both these factors should be highly conducive to "slow response" activity (Cranefield et al, 1971). No definitive study of the use of specific fast and slow channel blocking agents during acute ischaemia has been performed to date, however.

An alternative mechanism of slowed conduction is that of depression of excitability (Cranefield et al, 1973). Phase 4

diastolic depolarisation that fails to reach threshold can profoundly depress excitability and conduction (Singer et al, 1971). Preparations of ventricular muscle from diseased hearts in man (Singer et al, 1971) and the cat with low rates of diastolic depolarisation have low conduction velocities. Slow diastolic depolarisation was never observed in ischaemic epicardial tissue in this study, however.

The effect of anoxia and ischaemia in shortening the action potential is well known (Trautwein et al, 1954; McDonald and Macleod, 1973). These changes were clearly shown in the present study, but occurred more rapidly than in studies of anoxic myocardium or perfused pig heart (Downar et al, 1977). The rate of action potential shortening was particularly prominent after coronary occlusions that eventually resulted in ventricular fibrillation. Action potential shortening after three minutes of ischaemia was thus 50 to 60% of control and comparable to the degree of shortening achieved after 30 minutes of anoxia in isolated muscle preparations. The relationship of this action potential shortening to potassium depolarisation, pH changes, potassium fluxes and dependence of the slow inward current on glycolytic ATP production has been discussed (Section 2). Parallel changes in cellular ATP content and action potential duration have been shown (Cheneval et al, 1972; Hyde et al, 1972), in isolated tissue. Such information is not at present available for ischaemic tissue and the relative contribution of changes in cellular metabolism vis a vis passive ionic shifts on action potential duration are unknown. At slower heart rates and therefore presumably with milder ischaemia, transient prolongation of the action potential was noted during the initial

30 seconds after coronary occlusion. The mechanism of this effect could be related to that of the greatly prolonged potentials demonstrated in chronically ischaemic conducting tissue (Friedman et al, 1975). Alternatively, similar small effects of action potential prolongation have been demonstrated by extracellular acidosis (Fry et al, 1978) which is rapidly initiated during ischaemia (Williamson et al, 1976). It is possible, therefore, that opposing mechanisms are concurrently operative on determining action potential duration during ischaemia.

A consistent finding in severe ischaemia was the appearance of electrical alternans preceding or in association with arrhythmogenesis. Total cellular unresponsiveness as described in the pig after 12 to 15 minutes of ischaemia (Downar, 1977) was not seen. Alternans of action potential duration and amplitude occurred and was particularly prominent during "slow response" morphology potential activity. On occasion alternans of baseline DC diastolic level was observed. Intracellular potential changes were paralleled by alternating activity in the T wave and ST-segment of the surface epicardial electrocardiogram. Alternans of notches or morphology of the QRS complex accompanied more severe ischaemia. Similar alternating phenomena have been described during ischaemia (Downar et al, 1977) and in association with arrhythmias (El-Sherif et al, 1976). With increasing duration of ischaemia alternating activity progressed to overt 2:1 or 3:2 or more variable patterns of conduction block as recorded in intracellular, but not extracellular electrodes. Electrical alternans could arise as a true cellular potential alternans (Kleinfeld and Stein, 1968), either of potassium fluxes of repolarisation or of slow channel

calcium flux (Schneider and Sperelakis, 1974). This latter process is observed in vitro during anoxia and is thought related to intracellular ATP levels. Alternatively, alternans could result from an electrotonic interaction between areas of intermittent conduction block (Hellerstein and Liebow, 1950), possibly due to alternation of regional refractoriness (Janse and Downar, 1977) in areas showing prolonged or post-repolarisation refractoriness. Prolongation of refractoriness beyond the interdiastolic interval was indeed observed in some studies resulting in 2:1 block. Depressant effects of this order have been demonstrated on diastolic excitability threshold in the dog after administration of potassium (Elharrar et al, 1977) and by a postulated "ischaemic depressant factor" in local venous effluent blood on isolated pig right ventricular myocardium (Downar et al, 1977). Alternatively, an energy dependent electrical uncoupling effect at the level of the intercellular nexus has been proposed (Wojtczak, 1977).

The incidence of electrical alternans with respect to the onset of ventricular fibrillation showed a progressive increase until at 30 seconds prior to fibrillation, 95% of recordings showed alternans and 65% local conduction block. Alternating electrical activity could be of major importance in arrhythmogenesis. Asynchronous activation of adjacent groups of cells could rapidly lead to asynchrony and fractionation of conduction and localised zones of conduction block and unequal refractoriness, all variables highly conducive to re-entrant re-excitation. In particular, simultaneous focal re-entry at multiple sites would be enhanced.

Observations during successive occlusions at varying heart rates confirm the findings of other workers (Han, 1969; Kent et al, 1973)

that the vulnerability of the heart to ventricular ischaemia is enhanced with increasing heart rate. Even after high coronary occlusion ventricular fibrillation did not occur at rates below 140 beats per minute. At higher rates the incidence of fibrillation was increased and the time to onset of fibrillation following occlusion was decreased. Changes in action potential duration and endocardial-epicardial conduction were correspondingly more severe. The effect, however, of increasing severity of ischaemia with increasing heart rate, by virtue of enhanced oxygen demand and impaired coronary flow, must be counterbalanced against a rate-dependant electrophysiological effect. In the non-ischaemic heart the ventricular fibrillation threshold is increased (Han et al, 1966) and dispersion of refractoriness reduced by increasing heart rate. In addition the difference in action potential duration between Purkinje tissue and endocardial and epicardial myocardium is almost completely abolished at faster rates (Moore et al, 1964), and the relative refractory period and vulnerable period of the myocardium are shortened (Surawicz, 1971). El-Sherif et al (1976), however, noted a dual time-course of response to transient changes in heart rate following anterior septal arterial occlusion in the dog. An immediate and reversible effect of induction of high degrees of conduction delay and block of septal potentials was accompanied by a slower component of gradually and progressively increasing fragmentation of ventricular activation and induction of ventricular premature beats.

Re-entry

Data reported in this study are highly suggestive that re-entrant mechanisms, rather than enhancement of automaticity, are

operative in arrhythmogenesis in the acute phase of ischaemia. In no study was automatic activity observed, nor were delayed diastolic depolarisation, slow late diastolic depolarisation, oscillating after potentials or automatic slow potential activity. No sequence of arrhythmia suggested accelerated pacemaker activity. By contrast the findings of delayed conduction, fragmentation of conduction, divergence of refractoriness, action potential shortening and intermittency of conduction block fulfil all the major criteria for re-entrant re-excitation (Moe, 1975).

The exact nature of the re-entry circuit, if indeed it exists, must remain speculative. The original concept of re-entry as proposed by Schmitt and Erlanger (1928) and later expanded by Wit et al (1972) and others was based upon a fixed geometric circuit. Studies were performed on closed loops of Purkinje tissue locally depressed by elevations of extracellular potassium (Wit et al, 1972) or an isolated canine papillary muscle - false tendon preparations (Sasyniuk and Mendez, 1973). Although such reproducible re-entrant circuits may be operative in the more stable state of chronic ischaemia (Wit et al, 1974) or recurrent ventricular tachycardia (Wellens et al, 1974), it is unlikely that such a simple concept is valid during acute ischaemia. A complex functional dissociation of conduction exists which may change rapidly from moment to moment. Multiple potential re-entrant pathways may then co-exist simultaneously. El-Sherif et al (1976) suggest that the physical pathway of any re-entrant impulse may change from beat to beat. If asynchrony of intermittent conduction for example is widespread, then activation may pass via varied populations of cells on alternate beats. Conduction may be sufficiently delayed in one or more of these pathways

to initiate one or more re-entrant loops. Focal zones of conduction block could allow large temporal differences in activation on either side of the blocked zone to account for the widely disparate local conduction delays recorded. Intermittent partial relief of block could then excite a re-entrant circuit. Although premature beats on occasion preceded fibrillation, in general there was no such initiating impulse. The emergence of such a premature beat may then be necessary to, rather than causative of, induction of fibrillation. In keeping with the findings of other workers (Williams et al, 1974; Lie et al, 1975; El-Sherif et al, 1975) no discrete early vulnerable zone in diastole was demonstrable. Fibrillation arose late in diastole in many studies in keeping with the observation of late ventricular activation.

Automatic activity cannot be entirely exluded, however. It is practically impossible to detect a single ectopic firing focus in vivo. In addition currents of injury created by non-uniform diastolic depolarisation and recovery of excitability could generate automatic firing by 'focal re-excitation' (Han, 1972; Kleber et al, 1978).

The weight of evidence would favour re-entrant mechanisms. It follows therefore that any measure, pharmacological or metabolic, which ameliorates electrophysiological abnormalities relating to propensity to re-entry should have some beneficial effect on the propensity to arrhythmogenesis.

Metabolic effects

Associated metabolic effects of ischaemia (Owen et al, 1969; Opie, 1975) have been confirmed. Blood sampling from local coronary

venous effluent blood has shown potassium and lactate release (in terms of a negative arterial-venous difference) and a more positive arterial-venous difference of glucose following coronary occlusion. Although directional changes of metabolite release may be valid, caution must be exercised in quantitation of arterial-venous difference data due to variable inequalities of local venous admixture of blood from adjacent normal and ischaemic tissue. Even within ischaemic tissue heterogeneity of sampling must arise from effluent from heterogeneously perfused closely adjacent cells.

Nevertheless, data suggest a relative increase in glucose metabolism during moderate ischaemia of a similar order to that reported in other studies in the dog (Brachfeld and Scheuer, 1967; Owen et al, 1969; Opie et al, 1973; Norris et al, 1978). Enhanced glucose uptake may imply enhanced glycogen synthesis or enhanced glycolysis. Major glycogen breakdown has been demonstrated in the first 10 minutes of acute ischaemia in the dog (Opie et al, 1973). Indeed, breakdown of endogenous glycogen is thought to be the major source of lactate produced by accelerated glycolysis in the ischaemic zone. Considerable residual oxidative metabolism of glucose probably largely accounts for the increased arteriovenous differences observed. Both oxygen extraction ratios and $^{14}CO_2$ formation from $^{14}C$-glucose are enhanced at this time (Opie, 1976). The extent of acceleration of glycolysis is probably mediated by increased phosphofructokinase activity stimulated by rapid breakdown of ATP and increase of inorganic phosphate and ADP (Gudbjarnason et al, 1970) and little affected by associated rapid changes in intracellular pH (Neely et al, 1974). The lack of any significant observed alteration in arteriovenous FFA concentration differences across the ischaemic zone do not necessarily

imply an absence of reduced FFA oxidation. Substrate concentrations were low and an observable effect would need to be proportionately greater. Opie et al (1973) could show a significant reduction in FFA arterio-venous differences only after 20 minutes of ischaemia and then only of 10 $\mu Eq.l^{-1}$. In addition re-esterification of FFA to triglyceride is accelerated within the cell (Wartman et al, 1956) to further decrease FFA available for oxidation. The resultant balance of glucose and fatty acid oxidation is an important determinant of cytosolic ATP availability to power metabolically active electrophysiological processes (Corr, 1978).

Effect of successive occlusions

An important pre-requisite for assessment of an intervention during acute myocardial ischaemia is that of a reproducible model (Oliver, 1972). Unpaired studies have not been possible due to enormous individual variations in ischaemic response to the sheepdog. It was necessary, therefore, to perform a series of control coronary occlusions at fixed heart rate.

Of practical importance was the finding that in severe ischaemia electrophysiological measurements were different during the first compared with subsequent occlusions. Thus in dogs which developed ventricular fibrillation, the mean time of onset of this arrhythmia occurred 45 or more seconds earlier during the first than during subsequent occlusion. Similarly, after later occlusions, a slower rate of shortening of action potential and prolongation of conduction delay, together with less ST-segment elevation was observed despite comparable heart rates. These findings suggest enhanced vulnerability of the myocardium to arrhythmias during the initial

coronary arterial occlusion and are consistent with an altered ischaemic response in the relevant area of myocardium during successive periods of occlusion. This phenomenon is compatible with the finding of diminished contractility in myocardium reperfused after transient (5 - 10 minutes) ischaemia (Vatner et al, 1975; Smith and Kent, 1976). If ischaemia damages the contractile apparatus, a situation of reduced oxygen demand, and hence less oxygen debt, could be created during a second or subsequent occlusion. Milder changes during a second or subsequent occlusion may reflect some adaptive mechanism controlling coronary blood flow, possibly related to greater vasodilator activity consequent upon release of vaso-active adenine nucleotides (Berne and Rubio, 1969). Alternatively, depletion of noradrenaline during the first occlusion or a diminished reflex response could alter the metabolic and electrophysiological responses to successive occlusions. In the light of this finding studies by other workers employing similar experimental models of repetative coronary occlusion should be critically reappraised. A sham occlusion is necessary at least in severe ischaemia to ensure subsequent electrophysiological reproducibility. Careful studies of the reproducibility of successive diagonal branch occlusions of the left anterior descending coronary artery on regional ST-segment elevation suggest, however, that under these conditions of milder ischaemia a sham occlusion is not indicated (Vik-Mo et al, 1978).

## 14. EFFECT OF GLUCOSE

Infusion of glucose sufficient to approximately double arterial levels of glucose in the normally perfused heart had no effect on ventricular vulnerability, refractoriness, conduction or action potential characteristics.

During myocardial ischaemia differential effects were observed according to the severity of ischaemia and between control and border ischaemic zones. Thus during mild or moderate ischaemia, during which blood flow to the ischaemic zone was of the order of 50% of normal, ventricular vulnerability and the incidence of spontaneous ventricular arrhythmias were reduced and changes in action potential duration, conduction delay and ST-segment elevation ameliorated. Beneficial effects on abnormalities of refractoriness were greater in the border than control zones of ischaemia. By contrast, the incidence of ventricular fibrillation following induction of more severe ischaemia by high coronary occlusion was uninfluenced by glucose administration. Alteration in action potential duration and conduction delay was unchanged after five minutes of ischaemia, although some transient beneficial directional effects were observed.

These findings are consistent with apparently contradictory experimental reports in the literature. Antiarryhthmic effects and reduction of ST-segment elevation following infusion of glucose or glucose-insulin-potassium regimes are reported following two-stage Harris ligation (Sodi-Pallares, 1963) or acute ligation of the left anterior descending coronary artery (Regan et al, 1967; Burke et al, 1969) and reduction of ST-segment elevation following occlusion of a diagonal or apical branch of this artery in the dog (Maroko et al, 1972; Opie and Owen, 1976; Ahmed et al, 1978) or baboon (Opie et al, 1975). The severity of ischaemia in these studies is difficult to define in the absence of flow data, but the degree of ST-segment elevation and absence of distortion of QRS morphology due to conduction disturbances would imply selection of a mild or moderately ischaemic preparation. Maroko and Braunwald (1972),

indeed, rejected from their series any dog showing ST-segment changes attributable to conduction abnormality. Norris et al (1978), on the other hand, could show no beneficial effect of glucose-insulin-potassium on ST-segment elevation following a relatively higher coronary occlusion. Mean regional myocardial blood flows were, however, 0.29 $ml.min^{-1}.g^{-1}$, contrasting with values of 0.51 to 0.65 $ml.min^{-1}.g^{-1}$ in the low occlusion group of this study.

Similarly contradictory metabolic findings have been obtained in the isolated perfused heart. Recovery in contractile function after a period of anoxia is enhanced following addition of 11 mM glucose to the perfusate in the rat heart (Hearse and Chain, 1972) and enzyme release during ischaemia suppressed (Opie, 1976). In the globally ischaemic heart, however, survival time is diminished by addition of glucose (Liedtke et al, 1976). It is of interest to speculate that these latter studies may relate more to the high occlusion than low occlusion series of dogs.

In view of the lack of electrophysiological effect of glucose in normal myocardium and the fact that beneficial effects on ventricular vulnerability were not reproducible by infusion of isosmolar concentrations of the non-metabolisable sugar mannitol, it is likely that the beneficial electrophysiological effects observed in ischaemia are related to an influence on metabolism, either directly in terms of ATP availability or indirectly in terms of ionic fluxes. A lack of effectiveness of mannitol infusion has been demonstrated in studies during both regional ischaemia (Burke et al, 1969; Ahmed et al, 1978) and hypoxia (Apstein et al, 1976), although in higher concentrations, not attained in this study, protective effects have been observed (Powell et al, 1976)

related to osmotically induced reduction of cell swelling. Prolonged administration of hypertonic mannitol, however, induces release of lysosomal acid phosphatase even in the normal heart (Ali et al, 1977). The short duration of infusions and absence of altered plasma osmolality render these effects highly unlikely in this study.

The possibility of a 'polarising' effect of glucose by stimulating potassium uptake was suggested by Sodi-Pallares (1962). Although absolute membrane potentials were not determined in micro-electrode studies, the onset of 'slow response' type potentials was delayed during ischaemia with glucose. A maintenance of resting transmembrane potential for a longer period of time at a level below 60 mV at which fast channel inactivation occurs thus is suggested. A direct polarising effect need not be postulated, however, as an increase in ATP availability could delay potassium loss by stimulating the $Na^+$ -$K^+$ pump (Schwartz et al, 1975) or lead to activation of the electrogenic sodium pump, and so maintain resting transmembrane potential in the face of an elevated extracellular potassium concentration (Thomas, 1972). Certainly potassium loss from the ischaemic zone is reduced by glucose (Burke et al, 1969), although not within the first 15 minutes of ischaemia. No alterations in arteriovenous potassium differences due to glucose were demonstrated in the electrophysiological studies presented, although even large effects may have been observed by delayed washout or venous admixture effects. Arterial potassium levels were slightly reduced and could have contributed to a diminished extracellular potassium concentration in the ischaemic zone. A fall in tissue levels of potassium can be delayed by glucose post-infarction (Jennings et al, 1957). The reduction of delayed conduction observed after glucose could be accounted for by

such delays in loss of membrane potential and onset of slow conducting 'slow response' activity.

A more plausible explanation derives from the demonstration in isolated guinea pig ventricular muscle of a dependence of electrophysiological membrane properties on ATP derived from anaerobic glycolysis (McDonald and MacLeod, 1971; Section 5, Introduction). The glucose-induced reduction of action potential shortening during acute ischaemia of moderate severity could relate to the reduction of action potential shortening demonstrated in anoxic muscle. If so, both effects could be related to a stimulation of glycolytic ATP production and enhancement of slow inward calcium flux (Schneider and Sperelakis, 1974). Glycolytic ATP can be produced through anaerobic or aerobic glycolysis and production is proportional to the rate of glycolytic flux from endogenous and exogenous substrate (Neely and Morgan, 1974). Greater effects on normalisation of the duration of the action potential should occur, therefore, in less severely ischaemic zones of myocardium with greater capacity for stimulation of glycolysis. This was indeed observed. Normalisation of action potential duration occurred in two dogs following a diagonal branch occlusion inducing mild ischaemia. Normalisation of refractory period shortening was obtained after 30 minutes of mild ischaemia. With worsening ischaemia, in terms of greater action potential shortening with time, glucose was only partially effective in normalising changes. It is interesting to speculate that the transient beneficial effects of glucose on conduction and action potential duration following high proximal coronary occlusion might relate to the transient acceleration of glycolysis before end-product inhibition in the globally ischaemic rat heart (Neely et al, 1975). Secondary

effects of enhanced glycolytic metabolism also influence the action potential. Diminution of the fall in intracellular pH (Kohlhardt et al, 1976) and myocardial potassium loss (Weidman, 1956) influence action potential duration. Conversely, accumulation of lactate, as in severe ischaemia, depresses the recovery of action potential duration produced by glucose during anoxia (Opie, 1978).

Even within the moderately ischaemic region of myocardium produced by low or distal coronary occlusion, a spectrum of tissue oxygenation must exist related to local perfusion. It is of interest, therefore that determinations of gradients of refractoriness revealed more beneficial changes in the border zone than in the central ischaemic zone after glucose, determinations of refractoriness being performed within a few seconds of each other. Absolute changes in refractoriness in this series were small, supporting the concept of mild ischaemia. Regional myocardial blood flow was reduced to 60% of normal, and was not altered during ischaemia by glucose.

The effect of reduction of ventricular vulnerability or VPB threshold by glucose following low coronary occlusion must relate to the amelioration of alterations in local refractoriness and conduction velocity in the ischaemic zone. Normalisation or partial normalisation of these variables must reduce the potentiality for re-entrant excitation (Wit et al, 1975). The degree of severity of ischaemia at which glucose administration may be ineffective against arrhythmogenesis remains undefined. VPB threshold determinations were not possible following high coronary occlusion due to excessive induction of ventricular fibrillation by extra stimuli. The incidence of ventricular premature beats during milder ischaemia was reduced, however.

A metabolic basis for these electrophysiological effects at least in the low occlusion group of animals is suggested by the finding of an increased metabolic gradient of glucose between arterial and local venous blood before and during ischaemia. Similar values were found by Opie and Owen (1976) following glucose-insulin-potassium infusion. Although a regional arterial admixture abnormality could be postulated between ischaemic and non-ischaemic myocardium, the finding of no alteration in the regional distribution of blood flow following glucose infusion in either ischaemic or non-ischaemic myocardium would suggest that increases in arterial-venous gradients reflect increases in myocardial glucose uptake.

Elevations of substrate levels of glucose are well known to stimulate glucose metabolism at the expense of β-oxidation of free fatty acids (Opie, 1968; Neely and Moran, 1974) in normal myocardium. The increased arteriovenous glucose difference during glucose infusion in all series of studies would confirm this general shift in metabolism. The relative contribution of glucose and free fatty acids to metabolism, although not of importance in determining total energy supply under normal conditions, may be of great importance during ischaemia (Opie, 1970). These changes have been shown to be associated with an acceleration of anaerobic metabolism of glucose and an increase in aerobic glucose metabolism relative to that of free fatty acids (Opie et al, 1973). The further increase in arterio-venous difference of glucose in ischaemia during glucose infusion would suggest a further increase in anaerobic glycolysis and of aerobic glucose metabolism relative to fatty acid metabolism. This is supported by the absence of any increase in lactate arterio-venous difference during ischaemia. If lactate release is related to cellular lactate production, then stimulation

of aerobic rather than anaerobic metabolism is suggested. Tissue lactate accumulation is rapid, however, in the first few minutes of ischaemia (Braasch et al, 1968) and the release kinetics of lactate are considerably slower than the uptake kinetics for glucose (Brachfeld and Scheuer, 1967). This phenomenon could therefore merely represent a phase-lag of lactate release associated with considerable anaerobic glycolysis. The level of ATP production purely from anaerobic glycolysis, however, would be insufficient to account for major beneficial effects (Opie, 1970). Furthermore, accumulation of lactate and hydrogen ions interfere with oxidation of NADH causing accumulation of the nucleotide and inhibition of glycolysis (Rovetto et al, 1975). Transient acceleration of glycolysis is rapidly followed by inhibition in the globally ischaemic rat heart (Rovetto et al, 1973), even though intracellular levels of ADP, AMP and inorganic phosphate are increased. Glycogen stores are consumed during this process (Rovetto et al, 1973). Administration of glucose (and insulin) by contrast increases glycogen stores in normal tissue (Villar-Pallasi and Larner, 1969) and has a glycogen-sparing effect in the border ischaemic zone of the baboon (Opie et al, 1975) or dog (Opie and Owen, 1976). Tissue glycogen levels were not determined in those dogs with moderate ischaemia in this study, but might have been expected to be less reduced during ischaemia after glucose. Increased tissue glycogen content correlates with histological damage (Reimer et al, 1973) and could buffer the cell against transient anoxia by supplying substrate from glycogenolysis for endogenous anaerobic glycolysis. A protective effect of increased myocardial glycogen stores has been shown in the anoxic rat heart (Scheuer and Stezoski, 1970).

Secondary metabolic effects of glucose infusion include insulin release and suppression of plasma FFA levels (Dole, 1956). Under pentobarbitone anaesthesia resting plasma FFA levels were low and not reduced further by glucose. Secondary effects on FFA uptake related to substrate concentration are thus excluded. Plasma insulin levels were not determined. Although no change in arterio-venous FFA differences were detected during ischaemia after glucose, it might be expected that rates of FFA oxidation were reduced (Opie et al, 1973). Studies with $^{14}$C-FFA during glucose infusion would be necessary to confirm this. An inhibition of fatty acid oxidation by enhanced glycolytic flux during ischaemia should have a twofold beneficial effect on energy metabolism. Firstly, a decrease in oxygen consumption might be expected (Mjøs, 1971; Mjøs et al, 1974). Secondly, a reduction in intramitochondrial fatty-acyl CoA concentrations might speed the rate of exchange of ATP from and ADP into the mitochondria by relief of inhibition of the adenine nucleotide translocase (Pande and Blanchman, 1971; Shug and Shrago, 1973). Similarly, a reduction in the "oxygen wasting" effects of fatty acids such as the triglyceride - FFA reesterification-lipolysis cycle (Opie, 1975) or of uncoupling of oxidative phosphorylation (Pressman and Lardy, 1956; Borst et al, 1962) might be expected.

All these beneficial effects of glucose during ischaemia resulting in enhanced ATP production or availability within the cytosol are dependent both upon partial oxygenation of the tissue and lack of total inhibition of glycolysis. An explanation for a possible differential metabolic effect in severe ischaemia may lie in the absence of adequate or partial oxygenation, and the accumulation of metabolites leading to an inhibition of glycolysis. Local venous

sampling was not possible in the high occlusion group of dogs due to the severity of reduction of flow. It is likely, however, that venous effluent and extracellular levels of lactate and potassium and hydrogen ion were high. Inhibition of glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase, attributable to build up of lactate, NADH and hydrogen ion can be demonstrated following global ischaemia in the swine heart or rat heart following excess glucose and insulin (Liedtke et al, 1976), together with greater reductions in mechanical performance. Glycolytic intermediates, such as fructose-1-6-diphosphate, appear in higher concentrations during ischaemia after glucose. In the regional ischaemia studies of Opie (1975) and Opie and Owen (1976) in dog and baboon glucose did not influence tissue high energy phosphate or glycogen levels in the central infarcted region.

Alternative explanations for the observed phenomena require consideration. Glucose infusion may expand extracellular volume following an elevation in plasma osmolality. An increase in cardiac output, stroke volume, left ventricular end-diastolic pressure and left ventricular stroke work might then increase myocardial oxygen demand (Braunwald, 1971) and increase the severity of ischaemia. This is the likely explanation for the negative clinical study of Heng et al (1977) who gave glucose in large volumes in patients with acute myocardial infarction. Alternatively, an increase in plasma osmolality has been shown to be protective against myocardial ischaemic injury by improvement of coronary perfusion (Willerson et al, 1972). Elevations of osmolality of the order of 20 mS are required. Small volumes of glucose were given over short periods of time in this study and plasma osmolality was unaltered. Isosmolar infusions of mannitol

were used as a further control against possible non-specific effects on osmolality or plasma volume which might influence the VPB threshold. Values obtained during mannitol infusion did not differ from those obtained during control infusion of small volumes of saline. It would seem unlikely, therefore, that these mechanisms were operative.

There is evidence that elevation of plasma glucose may reduce the reflex sympatho-adrenal response to myocardial ischaemia. In a series of patients in the first two to three days following myocardial infarction, urinary catecholamine excretion is diminished by glucose-insulin-potassium infusion (Ceremuzynski et al, 1978). An antiarrhythmic effect would be expected from an inhibition of adrenaline or noradrenaline release immediately following acute experimental coronary occlusion. By contrast, however, carbohydrate feeding has been shown to increase the sympathetic activity to the heart (Young and Landsberg, 1976).

## 15. EFFECT OF INHIBITION OF LIPOLYSIS

Inhibition of lipolysis was achieved by combined infusion of isoprenaline and nicotinic acid. Significant reductions in arterial free fatty acid and glycerol levels were achieved in the presence of isoprenaline without effect on recorded haemodynamics or coronary blood flow. Changes were of a similar order to those associated with a reduction of myocardial oxygen consumption and a myocardial protective effect during acute ischaemia in the dog (Mjøs, 1971; Kjekshus and Mjøs, 1972, 1973; Mjøs et al, 1974).

Electrophysiological effects during acute ischaemia were in general less marked than following glucose infusion in the comparable 'low occlusion' group of animals. Significant reductions of ST-segment

elevation were observed, but changes in refractoriness, gradients of refractoriness, action potential duration and endocardial-epicardial conduction were of a minor nature. No effect on ventricular vulnerability, as assessed by VPB threshold determinations, could be observed. It is possible that an electrophysiological response relating to altered cellular metabolism and reduced uptake of free fatty acids could be partially masked by the response to high circulating isoprenaline levels. An attempt to differentiate between these two variables was made by assessment of ventricular vulnerability at one tenth of the isoprenaline infusion rate. An inadequate lipolytic response was obtained, however. High circulating catecholamine levels prevail, however, in the first hour of onset of symptoms of infarction in man (Vetter et al, 1974) and are probably largely responsible for the elevated circulating free fatty acid levels (Kurien and Oliver, 1970). It may be argued, therefore, that an experimental model with high basal catechol levels is comparable to the true clinical situation.

The striking reduction in ST-segment elevation during ischaemia accords with the findings of Kjekshus and Mjøs (1973) during diagonal branch occlusion of the left anterior descending coronary artery in the dog using the antilipolytic agent β-pyridylcarbinol, which is metabolised to nicotinic acid in the liver. Higher concentrations of isoprenaline (0.2 $\mu g.Kg^{-1}.min^{-1}$) were used, but similar reductions of free fatty acid obtained. ST-segment elevation during combined isoprenaline and β-pyridylcarbinol infusion was reduced to levels found in the control occlusion without isoprenaline infusion in the absence of change in heart rate, aortic pressure or coronary flow. Experimental studies with a variety of antilipolytic agents have confirmed this effect (Oliver, 1976; Miller et al, 1976; Mjøs et al, 1976;

Riemersma et al, 1977), as have clinical studies in man within the first six hours of onset of symptoms of infarction using both β-pyridylcarbinol (Kjekshus, 1976) and 5-fluoro-nicotinic acid, a nicotinic acid derivative without haemodynamic effects (Russell and Oliver, 1978).

The more obvious effects on ST-segment elevation than on local epicardial electrophysiological properties could relate to a greater contribution of the more ischaemic subepicardial or endocardial tissue to the current of injury. Endocardial-epicardial conduction time was delayed by only 10 mS, suggesting a milder degree of ischaemia than in the 'low occlusion' glucose treated animals. Epicardial regional myocardial blood flow in the ischaemic zone was 58% of normal. The presence of a small beneficial effect on action potential duration, but not on refractory periods during ischaemia, probably relates to the greater variability of refractory period determinations, post-repolarisation refractoriness being observed at some sites. A beneficial effect at such a site would be a shortening and not a prolongation of refractoriness. Gradients of refractoriness were diminished, however, after 7½ and 10 minutes of ischaemia, consistent with a small antiarrhythmic effect (Rowe et al, 1975).

A metabolic explanation for these changes could be provided by the known maintenance of action potential duration by glycolytic ATP (McDonald and McLeod, 1971). If antilipolytic therapy improved ATP availability to the cell membrane during ischaemia, a reversal of action potential shortening should result. Addition of palmitate to the moderately hypoxic guinea pig heart exacerbates action potential shortening (Cowan and Vaughan Williams, 1977), probably by this mechanism. Such an explanation for the electrophysiological findings during ischaemia

may also be considered, however, following the exclusion firstly of a direct electrophysiological effect of nicotinic acid itself, or secondly of an indirect effect on the severity of ischaemia by virtue of effects on coronary flow the inotropic state of the heart or preload or afterload to the heart (Braunwald, 1971).

The mode of action of nicotinic acid is not completely elucidated. It is known to inhibit hormone sensitive lipase in both adipose tissue (Butcher et al, 1968) and cardiac muscle (Christian et al, 1969) and to block the lipolytic effect of several hormones (Peterson et al, 1968) in adipose tissue. Accumulation of cyclic AMP is inhibited in adipose tissue (Butcher et al, 1968) and presumably also in cardiac muscle where some inhibition of the cyclic AMP sensitive lipase is operative. Some compartmentalisation of cyclic AMP response must be postulated to account for the lack of effect of nicotinic acid on contractility in the presence of isoprenaline in this and other (Kjekshus and Mjøs, 1972) studies. Alternatively, a different sensitivity to cyclic AMP-mediated inotropic and metabolic effects has been shown (Christian et al, 1969). An inhibition of glycerol release is obtained at $10^{-7}$M nicotinic acid in the perfused rat heart without effect on force of con traction. At higher concentrations ($10^{-5}$M) slight reduction in force of contraction occurs.

Nicotinic acid has no direct effect on the cardiac action potential (Beresewicz and Wojtczak, 1976) in isolated cat or calf ventricular muscle. No effects could be demonstrated in normal myocardium or on ventricular vulnerability or refractoriness in this study. A small prolongation of action potential of around 5 mS was recorded, however. This may have been artefactual relating to slight epicardial cooling or to effects of successive reperfusion.

A very small, but insignificant, trend to prolongation of action potential duration with successive occlusions occurred in the control series. Alternatively, a cyclic-AMP mediated effect under conditions of high catechol stimulation should be considered. Isoprenaline has the effect of inducing slight shortening of the action potential and refractoriness (Giotti et al, 1973; Tsien, 1973), probably by stimulation of the $i_x$ current of repolarisation following cyclic AMP dependent stimulation of the slow inward calcium flux. Nicotinic acid could thus partially reverse this effect by a direct action on reduction of intracellular cyclic AMP. The studies of Beresewicz and Wojtczak (1976) were not performed in the presence of any catecholamine. Mean refractoriness was prolonged by two to three mS by nicotinic acid, although this did not attain significant levels. It is unlikely that the mean prolongations in action potential duration of 9 to 11 mS during ischaemia could be entirely accounted for by this mechanism.

Although contractility, as assessed by the first derivative of left ventricular pressure, was not significantly reduced, mean values were slightly lower after nicotinic acid, both before and during ischaemia. Contractile response in the regionally ischaemic zone was not assessed. A small or regional negative inotropic effect cannot, therefore, be excluded. Preload, as assessed by left ventricular filling pressure, and afterload, as assessed by mean arterial blood pressure, were unaffected by addition of nicotinic acid. As in the studies of Kjekshus and Mjøs (1972), no effect was found on coronary blood flow.

The metabolic effects of antilipolytic therapy are well described (Oliver, 1975; Rowe et al, 1973; Luxton et al, 1976). In the presence of isoprenaline, the fall in arterial free fatty acid levels is associated with a reduction in myocardial fatty acid uptake

and a reduction of myocardial oxygen consumption of around 40% in the dog (Mjøs et al, 1974), the rat (Challoner and Steinberg, 1966) and in man (Simonsen and Kjekshus, 1978). In this study free fatty acid levels were halved and arterio-venous differences for free fatty acid reduced both before and during ischaemia, supporting the concept of a shift from fatty acid towards glucose metabolism. Almost 56% of the increase in myocardial oxygen consumption due to isoprenaline is attributable to the elevation of free fatty acids in the dog (Mjøs, 1971) and man (Kjekshus, 1977). Elevation of fatty acids to comparable levels using triglyceride-heparin produces similar increases in fatty acid uptake, but only a quarter of the increase in myocardial oxygen consumption (Mjøs, 1971). It is suggested that this discrepancy could be accounted for by a stimulation of intramyocardial lipolysis by isoprenaline. Glycerol release from the heart provides a reasonable index of intramyocardial lipolysis as glycerolkinase activity is low in the heart (Robinson and Newsholm, 1967) and oxidation of glycerol is insignificant (Lassers et al, 1972; Most et al, 1973). Nicotinic acid is known to inhibit glycerol release and intramyocardial lipolysis in the rat (Christian et al, 1969). In this study arterial levels of glycerol were reduced by nicotinic acid, suggesting inhibition of adipose tissue lipolysis. No significant release of glycerol from local venous blood could be demonstrated during isoprenaline infusion. Although lipolysis is stimulated by isoprenaline or adrenaline in the rat heart, in the absence of free fatty acid (Takenaka and Jakeo, 1976; Williamson, 1964), the stimulating effect of catechols on triglyceride mobilisation is abolished by addition of free fatty acids to the perfusate (Cross et al, 1975). Arterio-venous differences of glycerol after nicotinic acid did not differ significantly from zero before or during ischaemia.

Lowering of arterial fatty acid levels by inhibition of adipose tissue lipolysis may, therefore, have relieved an inhibition on intramyocardial lipolysis of endogenous triglyceride which was, in turn, inhibited by nicotinic acid. Similar findings were seen by Vik-Mo et al (1978) who noted reduced FFA extraction without alteration in glucose or lactate exchange following the antilipolytic agents nicotinic acid and sodium salicylate during regional ischaemia in the dog. This is interpreted as an effect of antilipolytic therapy on lowering α-glycerophosphate requirements for triglyceride synthesis and thereby making glucose available for immediate energy purposes.

An additional effect of nicotinic acid on reducing wasteful energy cycles within the myocardium is likely. On theoretical grounds, the oxygen consumption of hearts oxidising fatty acid rather than carbohydrate should be 10 to 15% higher (Opie, 1975). This is related to the production of two ATP molecules from oxidation of FADH generated by β-oxidation of fatty acids, in comparison to the three ATP molecules generated by oxidation of NADH. The figure in practice is closer to 40% (Kjekshus, 1976) and can be accounted for by generation of wasteful energy cycles.

Esterification of free fatty acid to triglyceride is stimulated during myocardial ischaemia (Scheuer and Brachfeld, 1966). If intramyocardial lipolysis is additionally stimulated as at low arterial fatty acid concentrations (Vik-Mo et al, 1978), then recycling of triglyceride can result. This effect is more likely if myocardial triglyceride levels are elevated. The oxygen consumption of ischaemic rat hearts is greater in hearts with higher triglyceride content and which show greater lipolytic activity (Brownsey and Brundt, 1977). Nicotinic acid should block this cycle by inhibition of lipolysis and

reduction of available fatty acid for re-esterification.

Similarly, a reduction in fatty acid uptake during ischaemia is thought to lead to a reduction in accumulation of free intracellular fatty acid or long chain fatty acyl CoA esters which may have direct toxic effects (Oliver, 1975), lead to uncoupling of oxidative phosphorylation (Borst et al, 1962; Challoner, 1966), or relieve inhibition of mitochondrial adenosine nucleotide translocase (Shug et al, 1975) and hence entrapment of ATP within the mitochondrion.

A small increase in cytoplasmic ATP by these mechanisms could account for the small electrophysiological effects observed by stimulation of the $Na^+$ $K^+$ pump and slow inward calcium flux (see Section 3).

## 16. GENERAL DISCUSSION

The studies described in this thesis support the concept of the potentiality to produce beneficial metabolic and electrophysiological effects during acute myocardial ischaemia by substrate manipulation. They suggest further, however, that these beneficial effects may only occur in mildly or moderately ischaemic tissue which may remain partially perfused and oxygenated.

The existence of a distinct "border zone" of partially oxygenated cells remains, however, controversial. NADH fluorescence studies in the ischaemic rat heart show the appearance of anoxic zones of the size of an arteriolar capillary bed with a very sharp border between anoxic and normoxic tissue (Steenbergen et al, 1977). Mitochondria in ischaemic tissue would appear to either demonstrate normal oxidative metabolism or switch off completely (Chance, 1976). The "border zone" could consist, therefore, of a heterogeneous mixture of well oxygenated and severely ischaemic cells with a sharp border

between (Morgan et al, 1977). Studies in the dog, by contrast, have shown a symmetrical profile of decreasing blood flow from the margin of the visibly cyanotic zone towards the central ischaemic zone, as assessed by autoradiography (Vokonas et al, 1978) or microsphere injection (Hearse et al, 1977). The width of the border zone in these studies was assessed to be 4.5 mm or 8 to 15 mm respectively, and to account for 30% or 37% respectively of the total ischaemic zone. Similar transitional "border" areas have been demonstrated histochemically (Cox et al, 1968) in metabolic studies (Opie et al, 1976) and electrographically (Bruyneel, 1975). Relatively large tissue slices were taken in studies failing to demonstrate such a border area (Marcus et al, 1975; Fischl et al, 1974). A model has been suggested, therefore, by Opie (1976), whereby an area of reduced blood flow might result in a separation of normoxic from "anoxic" cells by a zone of relatively hypoxic cells whose oxygen demand would be enhanced by increased work and effects of FFA and catecholamines. A state of partial oxidative metabolism is suggested by having half of the mitochondria in the cell anoxic and other half normoxic or, alternatively, by the mitochondria working at half-rate. Such a border area has been presumed to be the zone amenable to metabolic intervention (Maroko and Braunwald, 1972; Opie, 1966).

Apparently contradictory clinical findings, in particular regarding glucose or glucose-insulin-potassium intervention studies, may relate to a failure to distinguish between effects under conditions of varying degrees of severity of ischaemia, although interpretation is complicated by use of varying dosage regimes. Thus, Lesch (1974), in a study of patients with severe angina, found an increased incidence of

elevated left ventricular end-diastolic pressure, pulsus alternans, atrial fibrillation and ventricular premature beats during pacing induced ischaemia at plasma glucose levels of 20 mM. Using a lower dose of glucose (15 mM) and patients with less severe coronary artery disease, however, Chiong (1976) showed significant reduction in ST-segment depression and left ventricular end-diastolic pressure and increased lactate extraction during paining. Again, in studies within the first 12 hours of acute infarction, Heng et al (1977) showed a greater incidence of ventricular fibrillation in their glucose treated group, together with a significant increase in pulmonary wedge pressure and left ventricular stroke work index. High plasma glucose levels were attained, however, (30 mM) and effects could be attributed to an expansion of plasma volume. Rogers et al (1976), however, could show an almost 50 per cent reduction in mortality after glucose-insulin-potassium, with a smaller elevation of plasma glucose (12 mM). This beneficial effect was restricted to patients with milder ischaemic injury (as judged by the Pell or Norris index) and was more evident in patients with single vessel disease and no prior infarction. It was suggested that beneficial effects might only result under conditions of less severe coronary disease, together with adequate suppression of elevated plasma FFA levels and without induction of potentially harmful hyperosmotic effects. In earlier negative clinical studies (Mittra, 1965; Pentecost et al, 1968; MRC trial 1968) no attempt was made to distinguish between patient groups on the grounds of severity of ischaemia. Interpretation of these studies is additionally complicated by the lack of any consistent time of commencement of therapy and the variety of regimes and routes of administration used. A similar dichotomy of response has been reported

using sucrose as an antilipolytic agent between patients with smaller, as opposed to larger, infarcts, as measured by enzyme release within the first eight hours of symptoms (Tansey et al, 1979). Sucrose was effective as an antilipolytic agent only in those patients with smaller infarcts. Secondary effects of the degree of severity of ischaemia on the systemic metabolic response to ischaemia and the ease of substrate manipulation, therefore, must be taken into account.

If the degree of severity of ischaemia is indeed a determinant of the effectiveness of metabolic intervention, then the degree of ischaemia associated with induction of malignant arrhythmias may be of critical importance regarding practical anti-arrhythmic applications of substrate manipulation. An association has indeed been shown between arrhythmogenesis and the severity of myocardial ischaemia. The decrease in ventricular fibrillation threshold after experimental coronary occlusion correlates with infarct size estimated enzymatically and morphologically (Bloor et al, 1975). Similarly, the duration of ventricular dysrhythmias following infarction in man correlates with an estimated infarct size index (Cox et al, 1976). Correlations are, however, low ($r = 0.48$) and may not relate to an effect on the early ventricular dysrhythmias which show a different pattern of natural history (Oliver, 1976). In addition the speed of occlusion may be of greater importance than the size or ultimate degree of severity of ischaemia. A two-stage experimental coronary ligation is much less likely to induce fibrillation than a single ligation at the same site (Harris, 1950). The maximal heterogeneity of electrophysiological response required to initiate re-entrant activity need not equate with maximal severity of ischaemia with large areas of depressed or electrophysiologically unresponsive tissue.

Nevertheless, it might be expected that, at least to some extent, the greater the degree of myocardial ischaemic injury the greater the tissue available for electrophysiological disequilibrium and hence arrhythmogenesis. The beneficial electrophysiological effects reported in this thesis with glucose and antilipolytic therapy may reduce the vulnerability of the ventricle to development of malignant ventricular arrhythmias, but their effect on the incidence of the fatal arrhythmia of ventricular fibrillation in an individual case is less clear.

The findings of this thesis would suggest that a careful study in man of the electrophysiological effects of metabolic intervention within the acute phase of myocardial ischaemia, which takes into account the severity of myocardial ischaemic injury, may prove fruitful.

## 17. MAIN CONCLUSIONS

1. Normal cardiac electrical activity is metabolically dependent in terms of maintenance of transmembrane ionic gradients by ionic pumping mechanisms and maintenance of the plateau phase of the action potential by the slow inward current.

2. Many of the electrophysiological changes observed during acute myocardial ischaemia may be related to metabolic or biochemical changes, viz. ATP loss, potassium, hydrogen ion, lactate, catecholamine or "depressant" factor release.

3. A shift in myocardial metabolism occurs during ischaemia away from oxidative fatty acid metabolism towards oxidative glucose metabolism, but energy wasting systems develop.

4. The myocardial metabolic response to ischaemia may be modulated by alterations in substrate availability. The systemic response to ischaemia includes elevations of plasma free fatty acids and catecholamines which may induce an inappropriate metabolic response.

5. In general terms, it is suggested that glucose is "good" and free fatty acids are "bad" for the ischaemic myocardial cell. Metabolic intervention studies have shown protective effects on myocardial ischaemic injury and arrhythmogenesis from both elevation of plasma glucose and reduction of plasma free fatty acid levels. Many studies, however, have failed to confirm all these effects.

6. Hypothesis : It is suggested that substrate manipulation (elevation of plasma glucose or reduction of plasma free fatty acid levels) may ameliorate the myocardial metabolic response to ischaemia and hence diminish electrophysiological abnormalities of importance in the genesis of lethal arrhythmias.

7. In view of the probable relation between the early arrhythmic phase (Harris phase 1) following experimental coronary occlusion and the high incidence of sudden death immediately after onset of symptoms of myocardial infarction in man, the experimental model of acute coronary occlusion in the open chest anaesthetised dog was chosen.

8. Three experimental designs were adopted to allow for the combined measurements of metabolic gradients, regional myocardial blood flow and electrophysiological changes, viz:-

    a) Ventricular vulnerability to arrhythmogenesis - ventricular premature beat thresholds.

    b) Regional ventricular refractoriness.

    c) Intracellular and extracellular potential changes - including recording of epicardial action potentials, endocardial-epicardial conduction delays and ST-segment elevation.

A critical analysis and account of the development and validation of these techniques is given.

9. Acute coronary occlusion resulted in a phase of enhanced ventricular vulnerability to arrhythmias (reduced VPBT) between 2 and 7 minutes of ischaemia.

10. Differing patterns of change of ventricular refractoriness followed acute coronary occlusion in the ischaemic area. Shortening or prolongation or biphasic responses occurred. Shortening of refractoriness was more prominent in the less ischaemic peripheral area, whereas prolongation tended to occur in the more severely ischaemic central area.

11. Gradients of refractoriness were generated between normal and ischaemic zones and within the ischaemic zone which reverted to normal on reperfusion.

12. Spontaneous ventricular fibrillation was related to the degree of dispersion of refractoriness immediately preceding fibrillation and following release of occlusion.

13. Acute coronary occlusion resulted in transient APD prolongation followed by progressive shortening with progressively increasing endocardial-epicardial CT. Changes were enhanced by increased heart rates.

14. Ventricular fibrillation occurred at a time of maximum AP shortening, maximum CT and was preceded in 95% of instances by electrical alternans, variable conduction block (65%) and by potentials of "slow response" type morphology.

15. The data relating gradients of refractoriness, conduction delays and conduction block to ventricular fibrillation are highly suggestive of re-entry as an important pathogenetic mechanism.

16. Control studies of the effects of successive short coronary occlusions showed that electrophysiological changes following a proximal occlusion were greater during an initial, as opposed to subsequent, occlusions. Studies with metabolic intervention were therefore performed following an initial sham occlusion.

17. An increase in substrate availability of glucose by elevation of arterial glucose concentrations caused:-

    a) A reduction in ventricular vulnerability to arrhythmias (increased VPBT) between 2 and 7 minutes of ischaemia, compared with values during control saline and mannitol infusions associated with significant increases in the arterial-local venous metabolic gradients of glucose. Arterial potassium levels were slightly reduced.

b) A reduction in shortening of refractory period after 7½ minutes of ischaemia in the central ischaemic zone and after 5 and 12½ minutes of ischaemia in the peripheral ischaemic zone, together with reduced gradients of refractoriness between normal and ischaemic zones of 2½, 10 and 12½ minutes of ischaemia. These effects were not attributable to any effect on regional myocardial blood flow and were associated with similar metabolic changes as in (a) above. Almost complete normalisation of refractoriness in the ischaemic zone by glucose was demonstrated in studies after 60 minutes of moderate ischaemia. Conduction changes were small and did not attain significance.

c) A transient delay in APD shortening and CT prolongation following a high proximal coronary occlusion without effect on the incidence of ventricular fibrillation within 5 minutes of coronary occlusion.

d) Sustained reduction of APD shortening and CT prolongation following a lower more distal coronary occlusion, inducing less severe ischaemia. These changes were associated with similar metabolic effects to (a) above and could not be attributed to any effect on regional myocardial blood flow distribution.

18. Infusion of isoprenaline during acute coronary occlusion resulted in an elevation of plasma free fatty acid levels by stimulation of peripheral lipolysis, an enhanced ventricular vulnerability to arrhythmias, shortening of refractory period before and during ischaemia and increased gradients of refractoriness both during ischaemia and on reperfusion.

19. A reduction in substrate availability of free fatty acids by reduction of plasma free fatty acid levels following inhibition of isoprenaline stimulated lipolysis resulted in:-

a) No detectable effect on ventricular vulnerability to arrhythmias (VPBT) during ischaemia at dose levels of isoprenaline of 0.1 and 0.01 $\mu g.Kg^{-1}.min^{-1}$.

b) No significant change in mean refractory periods in the ischaemic region.

c) Significant reductions in gradients of refractoriness after 7½ and 10 minutes of occlusion and on reperfusion.

d) Small, but significant, reductions in APD shortening (1 and 4 minutes of ischaemia) and in CT prolongation (4 and 4½ minutes of ischaemia) after a low coronary occlusion. A slight APD shortening occurred before occlusion. These changes were not attributable to any haemodynamic effect or alteration of regional myocardial blood flow in normal or ischaemic regions and were associated with reduced metabolic gradients of free fatty acid across the ischaemic zone.

20. It is concluded that:-

a) Electrophysiological changes occur within the first 10 minutes of experimental coronary occlusion preceding ventricular fibrillation which are highly suggestive of the genesis of re-entrant excitation.

b) Metabolic manipulation by alteration of substrate availability can ameliorate some of these electrophysiological changes in the absence of an effect on regional myocardial blood flow and in association with demonstrable alterations in the metabolic gradients of substrates across the ischaemic zone.

c) During the moderate ischaemia of a distal coronary occlusion elevation of plasma glucose appears a more effective intervention than antilipolytic therapy, but the latter studies must be viewed in the light of the increased background catecholamine activity.

d) Metabolic intervention was therapeutically ineffective during the severe ischaemia of a high proximal coronary occlusion.

e) This dependence of metabolic response upon the severity of ischaemic injury may explain, in part, some contradictory clinical and experimental findings following metabolic intervention and suggests that any future therapeutic application of metabolic intervention should take this factor into account.

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Appendix 1

## PUBLICATIONS

A part of the work presented in this thesis has appeared in the following publications:-


Russell, D.C., Oliver, M.F., Wocjtak, J. (1977) Combined electrophysiological technique for assessment of the cellular basis of ventricular arrhythmias. Lancet, ii, 686-688.

Russell, D.C., Oliver, M.F. (1977) Effect of altering free fatty acid and glucose concentrations during acute myocardial infarction. In: "The first 24 hours in myocardial infarction", pp 54-56, Ed. Kaindl, Verlag Gerhardt Witzstrock.

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Russell, D.C., Smith, H.J., Oliver, M.F. (1979) Transmembrane potential changes and ventricular fibrillation during repetitive myocardial ischaemia in the dog. British Heart Journal (In Press).

Russell, D.C., Smith, H.J., Riemersma, R.A., Oliver, M.F. (1979) Effect of glucose on transmembrane potential changes during acute myocardial ischaemia in the dog. (Submitted to European Journal of Clinical Investigation).

Appendix 2

## FIGURES


1. Ionic conductances underlying the cardiac action potential.

2. Characteristics of fast response and slow response action potentials.

3. A speculative outline of the immediate and reversible intracellular metabolic response to myocardial ischaemia.

4. Experimental preparation.

5. Experimental arrangement for ventricular fibrillation threshold determination.

6. Fibrillation control unit, current driver and pacing unit.

7. Experimental arrangement for ventricular premature beat threshold determinations.

8. Electrocardiographic recordings of induction of repetitive ventricular premature beats.

9. Comparison of ventricular fibrillation threshold and ventricular premature beat threshold.

10. Experimental protocol for VPBT studies.

11. Experimental arrangement for regional ventricular refractory period studies.

12. Strength-interval curves at varying extrastimulus pulse widths.

13. Experimental arrangement for combined intracellular and extracellular potential recording studies.

14. Relation of VPBT to VPB frequency following coronary occlusion.

15. a) Patterns of regional ventricular refractoriness following
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16. Divergence of regional refractoriness preceding ventricular fibrillation.

17. Patterns of refractoriness associated with ventricular fibrillation.

18. Action potential changes in ischaemic myocardium following coronary occlusion.

19. Electrical alternans preceding ventricular fibrillation.

20. Changes in action potential duration, endocardial-epicardial conduction delay and mean ST-segment elevation preceding ventricular fibrillation.

21. Effect of successive occlusions on ischaemic changes in action potential duration and endocardial-epicardial conduction.

22. Effect of successive occlusions on mean epicardial ST-segment elevation.

23. Changes in action potential, conduction, alternans and block preceding ventricular fibrillation.

24. Effect of varying heart rate on action potential duration and endocardial-epicardial conduction following coronary occlusion.

25. Effect of glucose and saline infusions on VPBT following coronary occlusion.

26. Effect of glucose and mannitol infusions on VPBT following coronary occlusion.

27. Effect of saline, glucose and mannitol infusions on metabolic gradients of glucose following coronary occlusion.

28. Effect of glucose infusion on arterial-local venous lactate differences following coronary occlusion.

29. Effect of saline, glucose and mannitol infusions on metabolic gradients of FFA following coronary occlusion.

30. Effect of saline, glucose and mannitol infusions on metabolic gradients of potassium following coronary occlusion.

31. Effect of saline, glucose and mannitol infusions on metabolic gradients of sodium following coronary occlusion.

32. Effect of glucose on regional ventricular refractoriness following coronary occlusion.

33. Effect of glucose on gradients of refractoriness following coronary occlusion.

34. Effect of glucose on action potential duration and epicardial conduction delay following high (proximal) coronary occlusion.

35. Effect of glucose on action potential duration and epicardial conduction delay following low (distal) coronary occlusion.

36. Effect of glucose on arterial and arterial-local venous differences of glucose, potassium, lactate and FFA following coronary occlusion.

37. Effect of inhibition of isoprenaline stimulated lipolysis with 5-fluoronicotinic acid on VPBT following coronary occlusion.

38. Effect of isoprenaline on regional refractoriness following coronary occlusion.

39. Mean changes in regional refractoriness following coronary occlusion during infusion of isoprenaline.

40. Mean changes in gradients of refractoriness following coronary occlusion during infusion of isoprenaline.

41. Effect of inhibition of lipolysis on regional refractoriness following coronary occlusion.

42. Mean changes in regional refractoriness following coronary occlusion during inhibition of lipolysis.

43. Mean changes in gradients of refractoriness following coronary occlusion during inhibition of lipolysis.

44. Effect of inhibition of lipolysis on action potential duration, endocardial-epicardial conduction, and epicardial ST-segment elevation following coronary occlusion.

Appendix 3

## TABLES


1. Comparison of properties of fast response and slow response potentials.

2. Effect of successive refractory period determinations on refractory periods at increasing extrastimulus current strengths.

3. Effect on refractory period (extrastimulus twice threshold) of 35 previous measurements at increasing current strengths.

4. Action potential durations at three left ventricular sites recorded over 50 successive impalements.

5. Effect of initial coronary occlusion on action potential duration and endo-epicardial conduction time.

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7. Effect of heart rate on action potential duration, endocardial-epicardial conduction time and incidence of ventricular fibrillation.

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9. Values of VPBT following coronary occlusion during saline and mannitol infusions.

10. Values of VPBT following coronary occlusion during glucose and mannitol infusions.

11. Values of VPBT following coronary occlusion in group A and group B dogs during glucose and mannitol infusions.

12. Metabolic effects following coronary occlusion during infusions of saline, glucose and mannitol.

13. Effect of infusion of glucose on ventricular refractoriness during acute coronary arterial occlusion.

14. Effect of infusion of glucose on ventricular gradients of refractoriness during acute coronary occlusion.

15. Effect of infusion of glucose on metabolic gradients following coronary occlusion during studies of regional refractoriness.

16. Effect of infusion of glucose on regional myocardial blood flow following coronary occlusion during studies of regional refractoriness.

17. Effect of glucose infusion on ventricular refractoriness and epicardial conduction time after 60 minutes of ischaemia.

18. Effect of glucose infusion on action potential duration, endocardial-epicardial conduction time and incidence of ventricular fibrillation following high coronary occlusion.

19. Effect of glucose on action potential duration and endocardial-epicardial conduction time following low coronary occlusion.

20. Effect of glucose on metabolic gradients across ischaemic myocardium during electrophysiological studies of low coronary occlusion.

21. Effect of glucose infusion on regional myocardial blood flow determined 3½ minutes after low coronary occlusion.

22. Effect of isoprenaline infusion on VPBT following coronary occlusion.

23. Metabolic gradients across ischaemic myocardium during infusion of isoprenaline and isoprenaline + 5-fluoronicotinic acid.

24. Effect of isoprenaline infusion on changes in regional refractoriness during and after coronary occlusion.

25. Effect of isoprenaline infusion on changes in refractory period gradients in the ischaemic zone during and after coronary occlusion.

26. Effect of inhibition of isoprenaline induced lipolysis by nicotinic acid on ventricular refractoriness during and after coronary occlusion.

27. Effect of inhibition of isoprenaline induced lipolysis by nicotinic acid on refractory period gradients during and after coronary occlusion.

28. The effect of inhibition of isoprenaline stimulated lipolysis by nicotinic acid on action potential duration, endocardial-epicardial conduction time and ST-segment elevation.

29. Effect of nicotinic acid induced inhibition of lipolysis on metabolic gradients across ischaemic myocardium during electrophysiological studies of low coronary occlusion.

30. Effect of inhibition of lipolysis on regional myocardial blood flow determined before and 3½ minutes after coronary occlusion.

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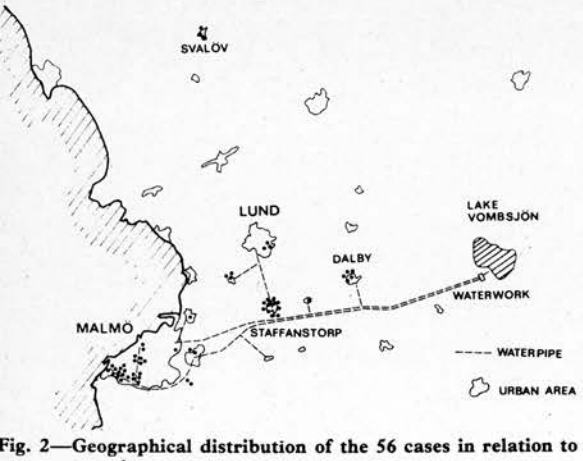


**Fig. 2—Geographical distribution of the 56 cases in relation to water-supply.**

1). No new cases were reported after May, 1976, and the severity of attacks in the old cases gradually decreased and symptoms had disappeared by the end of August, 1976.

53 of the 56 cases were in an area supplied with water from a lake (Vombsjön) approximately 30 km east of Malmö (fig. 2). The water from this lake is artificially filtered through large sand banks, a procedure taking 3 months, and then treated with ammonia and chlorine. The remaining 3 cases were reported from the village of Svalöv, where the water is obtained from deep-bore wells and is not treated with any chemicals. No cases were reported from neighbouring villages with other sources of water.

6 of the patients lived in flats, and the remaining 50 in their own houses. In all but 2 families only one member was affected. 52 of the patients were non-smokers. None of them had a history of allergy.

*Water Investigations*

Routine bacteriological and chemical tests of the water during 1975 and 1976 showed nothing abnormal. During the investigation of the outbreak a large number of water samples were obtained from various parts of the supply system. The concentrations of magnesium, sodium, chlorine, and ammonia were normal for drinking water. The calcium content was 68–136 mg/l, the nitrate content 7 mg/l, which although fairly high, is normal for this area. Gas chromatography (performed on 'Porapaque Q' column at 200°C and on 'Silikon Q' 2% OV 101 at 280°C on unconcentrated water and on water concentrated 100 times) did not show any extraneous substances.

The total bacterial counts were low, usually below 1000/dl. There were only minor differences in the counts between cold and hot water. The bacterial flora was dominated by gram-negative microorganisms, mainly *Alcaligenes, Pseudomonas,* and *Acinetobacter. Escherichia coli* or other microorganisms indicative of fæcal contamination were not found. Culture at 56°C did not grow any thermophilic microorganisms. Cultures for yeasts, moulds, and mycobacteria were negative. Water from neighbouring areas, where there had been no cases, served as a control. The chemical and microbiological findings in these water samples were similar to those mentioned above.

Lake Vombsjön is known to have a rich flora of blue-green algæ, but no algæ were found on microscopic examination of the samples of water taken at the waterworks, in the mains, or in the houses. Hot and cold water samples from the affected areas as well as from a control area were studied with the *Limulus* test for endotoxins.[2] There was no difference in the amount of endotoxin between the samples. Neither did inhalation tests in guineapigs with aerosolised water samples[3] show any difference in the endotoxin levels.

*Experimental Provocation*

In the patients a hot bath at home lasting 10–20 min was soon followed by a typical attack. The reaction was not related to any special type of soap, bath salts, or oil. A shower in the same bathroom usually did not cause any symptoms, except in a few very sensitive individuals. In a series of experiments carried out in susceptible volunteers we found that inhalation for about 10 min with the face just above the water without the person entering the bath was enough to provoke a reaction. Hot water elicited a reaction, while cold water did not. If the person inhaling wore a gas mask, there was no reaction. Susceptible individuals could take a bath outside the area supplied by the lake without being affected. Within the area, an attack could be provoked in susceptible individuals in many places, even in houses whose occupants did not react to a hot bath.

## Discussion

The epidemic was evidently caused by the inhalation of some agent in the water. The clinical picture was characterised by fever, leucocytosis, and an increase of C-reactive protein, all signs of an inflammatory reaction. But the attacks were brief, had an incubation period of only a few hours, and were self-limiting. They could be repeatedly provoked at short intervals. These features ruled out infection as a probable cause of the reaction.

The fact that there were only 56 cases reported in an area of some 350 000 inhabitants indicates that some kind of hypersensitivity must have been involved. Many outbreaks of "hypersensitivity pneumonitis" caused by inhalation of organic dust have been described.[4] Similar syndromes due to air-conditioning and humidifying systems contaminated with thermophilic *Actinomyces, Micropolyspora,* and protozoa (*Nægleria gruberi*) have been reported.[5-7] Recently another form of this condition has been described, where the provoking agent was *Pullularia* in a water bucket in a sauna.[8] In some respects the clinical picture in our series resembled that of "hypersensitivity pneumonitis". The immunoglobulins however, were not increased and chest X-rays never showed anything remarkable, perhaps because exposure was intermittent and brief. However, neither *Actinomyces* nor fungi, often involved in such reactions, could be isolated from the water, and there was no serological evidence in support of a bacterial or fungal origin.

Endotoxin from gram-negative bacteria may cause fever and can be absorbed from the respiratory tract in rabbits.[9] In our series, however, the bacterial counts were never ver very high and the amount of endotoxin as measured by the *Limulus* test and the guineapig inhalation test was low.

Similar febrile reactions may result after inhalation of metal fumes and certain plastic fumes (polymer fume

fever).[10] No evidence for such a mechanism was found in our series.

Blue-green algæ are known to produce toxins harmful to cattle.[11] Febrile reactions possibly caused by such toxins have been reported in connection with hæmodialysis.[12] In our series no algæ were found in the water. Theoretically, a toxin could have passed the sand filters and the interval of 3 months between the growth season of the algæ and the outbreak corresponded exactly with the time required for the water to pass through the filters. On the other hand, 3 cases were seen in an area supplied by subsoil water, in which algæ are not known to live.

During the investigation of this outbreak we learnt that similar cases had occured earlier in other parts of Sweden (some 30 cases between 1952 and 1975 in 11 different places in southern Sweden). The nature of the exposure and the clinical picture seem to have been identical with our series. The outbreak described here is evidently not unique, and the illness may not be as rare as supposed.

We thank Dr Gertrud Cronberg, Institute of Limnology, Universi of Lund, for examining algæ, Dr Göran Odham, Institute of Zo logy, University of Lund for gas chromatography, and Prof. Ragn Rylander, Institute of Hygiene, University of Gothenburg, for estim tion of endotoxin.

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# Preliminary Communications

## COMBINED ELECTROPHYSIOLOGICAL TECHNIQUE FOR ASSESSMENT OF THE CELLULAR BASIS OF EARLY VENTRICULAR ARRHYTHMIAS

### Experiments in Dogs

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***Summary*** A new technique has been used in dogs to make combined measurements in vivo of conduction delay, action potential, and epicardial ST-segment during myocardial ischæmia. These measurements should provide new information about ionic and metabolic cellular changes relating to the onset of ventricular arrhythmias.

INTRODUCTION

MALIGNANT ventricular arrhythmias occurring within the first few minutes of the onset of symptoms probably account for the majority of sudden deaths from myocardial ischæmia in man.[1] These probably correspond to the early phase of arrhythmias following coronary-artery occlusion in dogs.[2] An important mechanism in their genesis is believed to be re-entry,[3] whereby a cardiac impulse may be conducted more slowly round a region of unidirectional conduction block to excite tissue distal to the blocked site and then re-excite tissue proximally. Extracellular recording techniques have demonstrated striking delays in conduction[4,5] and variations in refractoriness[6] in acute ischæmia which would predispose to this process.

Knowledge of the relationship of intracellular potential changes in cardiac tissue to re-entry has been derived largely from studies in isolated tissue under simu lated conditions of ischæmia.[3] The relevance of suc changes to arrhythmogenesis can be studied unde physiological conditions only in the heart in situ. Direc intracellular recording during ischæmia is possible wit the floating microelectrode technique,[7–9] but no suc study has been directed primarily towards the factors ir itiating arrhythmias. We describe a combined electrc physiological technique with intracellular and extra cellular recording suitable for the study of arrhythmia during early myocardial ischæmia. We also present som preliminary findings.

EXPERIMENTAL PROCEDURES

Mongrel dogs are anæsthetised with pentobarbitone 3 mg/kg and maintenance anæsthesia of 3 mg/kg/min. Follov ing intubation and artificial ventilation, a left thoracotomy performed, and the heart is suspended in a pericardial cradl The left anterior descending coronary artery is dissected fre proximal to the main diagonal branches. A miniature hydrau ic-pressure occlusion cuff is applied loosely round the vessel t allow intermittent occlusion of the artery. Occlusion produce ischæmia in the antero-apical region of the left ventricle.

*Conduction Delay*

A copper wire plunge electrode insulated except at the tip inserted through the left ventricular wall into the endocardiu to detect conduction delay between endocardial and epicardia activation. The onset of the recorded electrogram coincide with the onset of ventricular endocardial activation.

*Action Potential*

Floating microelectrodes can be positioned with micro manipulators on a potentially ischæmic area of myocardium Before use, the distal shafts of glass microelectrodes are fille with potassium chloride, 3 mol/l, and the tips are suspende from fine silver wire. Two electrodes are applied close to on another, one as an intracellular electrode and the other as a extracellular indifferent electrode for differential recording c the action potential. The onset of epicardial activation is the given by the time of onset of the recorded action potential. I initial studies it was noted that more stable and prolonged ir tracellular recordings were obtained with thicker-walled glas (external diameter 2·5 mm instead of 2 mm, and internal d ameter 1 mm). By this means we have maintained cellula impalements for 5–10 min.

*Epicardial ST-segment Recording*

The degree of epicardial ST-segment elevation provides an independent index of myocardial ischæmic injury.[10] This is recorded from five disc electrodes (1·5 mm diameter) sutured to the epicardium at least 1 cm from the border area of ischæmia in the potentially ischæmic zone. By passing the electrograms through an averaging switch-box, a signal averaged from the input of five leads is obtained, and this may be recorded either from the oscilloscope or subsequently from tape. Constant heart-rate must be maintained by atrial pacing to eliminate rate-dependent influences.[11]

## RESULTS

A characteristic sequence of recordings following coronary-artery occlusion in a dog is shown in fig. 1, the cardiac action potential being recorded from the centre of the ischæmic zone. The notch on the upstroke of the action potential corresponds to the "extrinsic potential" of Prinzmetal et al.[8] and is related to the activation of tissue between the recording and indifferent electrodes.[9] By recording differentially from two closely applied floating microelectrodes, large extrinsic potentials are

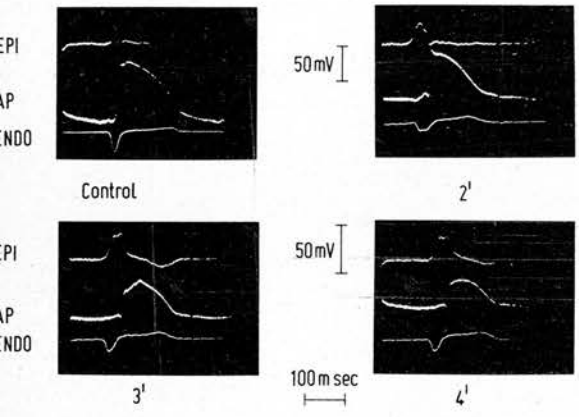


**Fig. 1—Recordings of action potential (AP) endocardial (ENDO), and averaged epicardial (EPI) electrograms from dog heart in situ before and 2, 3, and 4 min after coronary-artery occlusion.**

Note shortening of the action potential and progressive delay in its onset with respect to the endocardial-potential loss of the early rapid phase of depolarisation. The epicardial electrogram demonstrates the conduction abnormality and ST-segment elevation.

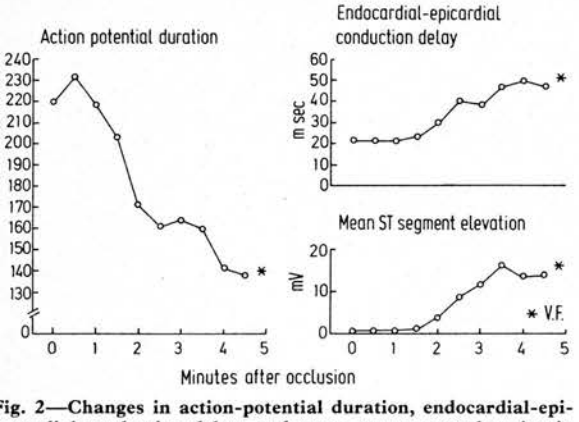


**Fig. 2—Changes in action-potential duration, endocardial-epicardial conduction delay, and mean ST-segment elevation in dog heart after acute coronary-artery occlusion.**

Ventricular fibrillation (V.F.) resulted after 4 min 50 s of ischæmia, at a time of maximum recorded conduction delay and action-potential shortening.

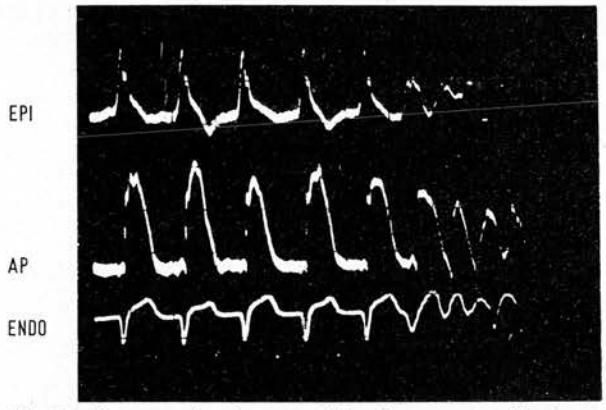


**Fig. 3—Alternans of action potential and ST-segment of epicardial and endocardial electrograms preceding ventricular fibrillation.**

Note the increased asynchrony between intracellular and extracellular recordings at onset of fibrillation.

eliminated. Endocardial activation can be seen to precede epicardial activation. After coronary occlusion action-potential configuration changes rapidly, with shortening, loss of amplitude, and disappearance of the initial rapid phase of depolarisation. Changes of the order of those seen in isolated superfused anoxic ventricular muscle after 30 min of total anoxia are observed in vivo after 2–3 min.[12]

More detailed findings from one dog are shown in fig. 2, in which ventricular fibrillation resulted after 4½ min of ischæmia. Within 30 s of occlusion, transient prolongation of action potential occurred and was followed by shortening and development of a more triangular shape. By 2 min endocardial-epicardial conduction was delayed, and mean epicardial ST-segments were elevated. At 4 min after occlusion, the action potential assumed an appearance similar to that of "slow response" potentials.[13] Such potentials occur with a reduction in transmembrane resting potential to less than −60 mV, at which voltage the rapid inward sodium current is inactivated.[14]

An important characteristic of such potentials is their slowness of conduction. Together with conduction delay, action potential alternans developed, with alternation of amplitude, duration, and morphology. Fig. 3 shows this phenomenon preceding ventricular fibrillation, together with its association with alternation of ST-segment elevation in epicardial and endocardial leads. In some dogs this progresses to varying degrees of localised conduction blocks, either 2:1 or more irregular patterns, before the appearance of arrhythmias. Arrhythmias were not observed, at least in our pilot studies, in the absence of either preceding electrical alternans, or abnormal potentials, or long conduction delays.

## DISCUSSION

The importance of the mechanism of re-entry in the genesis of arrhythmias with respect to the development of spontaneous ventricular automaticity[15] or facilitation by local catecholamine release [16] can be adequately studied only by direct intracellular recording in vivo. Action-potential recording from floating microelectrodes is superior to the monophasic action potential obtained

by induction of tissue injury with a suction electrode.[17] Transmembrane potential recordings and upstroke velocity may not, however, at all times represent true absolute values, because of unavoidable movement artefact and variations in electrode-tip potential, although the time of onset and action potential are exactly reproducible. The early transient prolongation of action potential after occlusion has not previously been reported. This may be akin to the prolonged potentials seen in chronically ischæmic conducting tissue.[18] We have not observed spontaneous ventricular cardiac muscle-cell automaticity in our acute ischæmic preparation, but localised conduction blocks, together with slow conduction and abbreviated action potentials, create the degree of asynchrony necessary for local re-entry. Temporal dispersion of repolarisation may also predispose to re-entrant activity. We propose to make further studies with intracellular recording from two or more ischæmic sites to delineate the extent of such asynchrony in adjacent tissue. We suggest that the relative importance of some ionic and metabolic cellular changes resulting in early arrhythmias may be elucidated with this combination of in-vivo measurements of conduction delay, action potential, and ST-segment current of injury. This technique may also be of value in examining the effect of metabolic or pharmacological interventions.

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## IS DEXTRAN 70 A LYMPHOCYTE MITOGEN?

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***Summary*** Dextran 70 ('Lomodex') produced significant increases in thymidine incorporation in a modified whole-blood method of investigating lymphocyte blastogenesis. This blastogenesis occurred at molar concentrations of $7\times10^{-6}$ and $7\times10^{-5}$. At $7\times10^{-7}$ mol/l dextran alone did not significantly increase thymidine incorporation; however, when this concentration of dextran was added simultaneously with phytohæmagglutinin there was a significant increase in incorporation. Conversely when dextran was added before P.H.A., thymidine incorporation was decreased. These variable effects indicate that dextran 70 may be a B-cell mitogen. Dextran 70 is widely used and these effects on lymphocyte blastogenesis may be clinically relevant.

### INTRODUCTION

DEXTRANS are now widely used in clinical medicine as plasma substitutes and as prophylaxis against deep-vein thrombosis. The dextrans used are predominantly linear and are thought to have no important effect on the immune system. However, dextrans with a high degree of branching have been shown to have mitogenic effects on human B lymphocytes.[1]

We investigated the possible mitogenic effect of dextran 70 ('Lomodex') by means of a modified whole-blood method which measures the effect on lymphocyte stimulation.[2] The effect of dextran 70 on the lymphocyte mitogenic response to phytohæmagglutinin (P.H.A.) was also investigated.

### METHOD

Defibrinated blood-samples were diluted 1/10 in medium TC199. 200 µl volumes were then distributed into microtitre plate wells. The dose response of dextran 70 was studied by making serial dilutions of the dextran from $7\times10^{-8}$ to $7\times10^{-5}$ mol/l. 16 replicate wells were used for each dilution. The cultures were incubated for 48 h and then labelled for 4 h with triated thymidine (5 µl of 0·5 µCi). Cells and medium were recovered from each well onto filter paper by means of an automatic multiple sample harvester. The filters were added to scintillation fluid and counted for one minute. Two experiments were performed on blood-samples from each of 3 subjects.

The maximum non-stimulatory dose of dextran ($7\times10^{-7}$ mol/l) was then used to investigate whether dextran had an effect on P.H.A. stimulation. P.H.A. (Wellcome purified PHA K 2313) at a concentration of 0·1 µg/µl was used. 7 sets of 32 replicate wells were then incubated for 48 h as before but the following were added to the wells for the times indicated: (a) P.H.A. present for the whole 48 h; (b) P.H.A. present during only the last 24 h; (c) dextran and P.H.A. both present for the whole 48 h; (d) dextran for 48 hours but P.H.A. for the last 24 h; (e) P.H.A. for 48 hours but dextran for the last 24 h; (f) dextran present for 48 h; (g) dextran present for the last 24 h Two experiments were performed on each of 3 subjects.

### RESULTS

Significant ($P<0·01$) increases in thymidine incorporation occurred with dextran 70 at molar concentrations of $7\times10^{-6}$ and $7\times10^{-5}$. At $7\times10^{-7}$ mol/l dextran 70 had no significant stimulating effect (table I). However stimulation was significantly increased when $7\times10^{-7}$ mol/l dextran was added simultaneously with P.H.A., and

TABLE I—EFFECT OF DEXTRON 70 UPON THYMIDINE INCORPORATION (MEAN±S.E.)

| Dextran 70 (mol/l) | d.p.m. | Significance |
|---|---|---|
| 0 | 6249±946 | |
| $7\times10^{-8}$ | 6164±1200 | N.S. |
| $7\times10^{-7}$ | 6438±756 | N.S. |
| $7\times10^{-6}$ | 14358±1365 | $P<0·01$ |
| $7\times10^{-5}$ | 23156±971 | $P<0·001$ |

# VENTRICULAR REFRACTORINESS DURING ACUTE MYOCARDIAL ISCHAEMIA AND ITS RELATIONSHIP TO VENTRICULAR FIBRILLATION

BY

**D. C. RUSSELL AND M. F. OLIVER**

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*Cardiovascular Research*, 1978, **12**, 221–227

# Ventricular refractoriness during acute myocardial ischaemia and its relationship to ventricular fibrillation

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SUMMARY Studies were made in the anaesthetised dog of the effects of repeated acute occlusions of a branch of the anterior descending coronary artery on ventricular refractory periods in adjacent ischaemic and non-ischaemic myocardium.

Differences occurred in refractoriness between normal and ischaemic areas in the ventricle. This was greatest 2.5 min after occlusion, and on release of occlusion, ventricular refractory periods reverted to normal within 5 min.

Spontaneous ventricular fibrillation was directly and significantly related to the degree of dispersion of refractoriness in a given dog immediately preceding release and following release of occlusion. Infusion of isoprenaline caused significant shortening of refractory period and increased dispersion of refractoriness during ischaemia.

Studies of dispersion of refractoriness should prove valuable in assessing the efficiency of metabolic or antiarrhythmic protection against ventricular fibrillation.

The vulnerability of the heart to arrhythmias and ventricular fibrillation is increased during acute myocardial ischaemia. An important mechanism is that of repetitive excitation by re-entry (Wallace and Mignone, 1966; Cranefield and Hoffman, 1971; Wit *et al.*, 1974) which, in the case of ventricular fibrillation, may lead to disorganised electrical activity (Surawicz, 1971). Although no single factor may predispose to re-entrant activity, an increase in dispersion of refractoriness across the myocardium may increase the likelihood of re-entry of an impulse from an area of longer refractory period to one of shorter refractoriness. Dispersion of refractoriness has been shown to be increased by ischaemia and catecholamine infusions (Han *et al.*, 1964) and to be related to a decrease in fibrillation threshold (Han and Moe, 1964). Conflicting reports exist, however, of the changes in ventricular refractoriness during ischaemia (Reynolds *et al.*, 1960; Tsuchida 1965; Han, 1969). The purpose of this study is to define the changes of ventricular refractoriness following acute coronary occlusion by simultaneous comparison in three discrete locations—one non-ischaemic and two ischaemic—and to examine their relationship to the onset of spontaneous ventricular fibrillation.

[1]Reprint requests to: Dr M. F. Oliver, Department of Cardiology, Royal Infirmary, Edinburgh.

## Methods

Studies were performed on 15 mongrel dogs (weight 17 to 25 kg) anaesthetised by pentobarbitone 25 $mg \cdot kg^{-1}$ and subsequently by 3 $mg \cdot kg \cdot h^{-1}$ infusion. Additional supplements of 3 $mg \cdot kg^{-1}$ were administered as required. Following intubation and under controlled ventilation with room air, the thorax was opened *via* the fourth intercostal space and the heart exposed and suspended in a pericardial cradle. A cannula was placed in the femoral artery for blood pressure recording.

A major marginal branch of the left anterior descending coronary artery was dissected free at its origin and a ligature placed loosely around it. A short test occlusion using a light spring clip was performed to delineate the potentially ischaemic area of ventricle. Pairs of fine insulated copper hook electrodes (0.13 mm diameter) were inserted in the subepicardium in each of three areas of the left ventricle, a non-ischaemic area (NA) of ventricle and in two potentially ischaemic areas, one at approximately the centre of the potentially ischaemic zone (central ischaemic area or CA) and one at the periphery adjacent to the apex of the left ventricle (peripheral ischaemic area or PA) (Fig. 1). The tips of these electrodes were 2 to 3 mm apart for bipolar stimulations. Pacing electrodes were attached

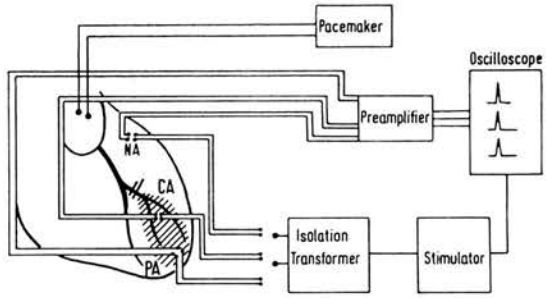


Fig. 1 *Schematic representation of the experimental arrangement for measuring ventricular refractoriness. Intermittent occlusion of the diagonal branch of the left anterior descending coronary artery was used to induce ischaemia (represented as the shaded area). Bipolar stimulating and recording electrodes were applied in non-ischaemic (NA) amd ischaemic central (CA) and peripheral (PA) areas. Measurements were performed at fixed heart rate by atrial pacing.*

to the left atrium for overdrive pacing. At least 30 min was then allowed to elapse for currents of injury to subside.

Bipolar electrograms from each of these three ventricular sites were displayed on a DM63 Tektronix oscilloscope. Overdrive atrial pacing was commenced to maintain constant heart rate at 200 beats·min$^{-1}$. Functional refractory periods (FRP) were determined at each site by the extra-stimulus method using a purpose-built stimulator with a variable delay linked to the oscilloscope sweep. Extra-stimuli of 2 ms pulse duration and 1.5 to 2 times threshold voltage were delivered at a known time interval after local depolarisation commencing in mid-diastole. This interval was progressively shortened by 5 ms decrements from mid-diastole towards the T wave and by 2 ms decrements on the T wave. Ten beats recovery period was allowed between each stimulus. FRP was defined as the time interval from the peak of the R wave of the bipolar electrogram at each individual site to the earliest stimulus producing a propagated impulse. Control measurements were taken over a period of 5 min to ensure reproducibility of results. Coronary clip occlusion was then performed and serial recordings of FRP performed at 2.5 min intervals in each of the three zones. Each series of readings could be performed in about 1 min and were performed in the same sequence on each occasion. The clip was released after 15 min and recordings of FRP were continued for a further 15 min.

An isoprenaline infusion 0.1 $\mu$g·kg$^{-1}$·min$^{-1}$ was commenced 40 min after the first occlusion and the heart again paced from the atrium (200 beats·min$^{-1}$). Recordings of FRP were similarly obtained before and during a further 15 min period of occlusion and following release. Arterial $Po_2$, $Pco_2$, and pH determinations were performed between occlusions. Blood pH in all cases remained in the range 7.35 to 7.45.

Episodes of ventricular fibrillation were managed by 15 Watt-sec d.c. countershock but measurements immediately following fibrillation and countershock were excluded from analysis in view of possible induced haemodynamic and electrophysiological effects.

Statistical significances were calculated using Student's $t$ test for paired data.

## Results

### REFRACTORY PERIODS

No significant change in FRP was observed either during the 10 min coronary occlusion or after release of the occlusion in the NA, in any dog.

By contrast a variety of responses were observed in the two ischaemic areas (CA, PA), representative examples being shown in Fig. 2.

Steady values for FRP were observed prior to occlusion, Following occlusion the following patterns emerged:

(a) A progressive decline in FRP with time (Fig. 2A).
(b) A sudden sharp increase of FRP to around 150% of control followed by a similar progressive decline (Fig. 2B).
(c) An initial fall of FRP followed by a rise (Fig. 2C).
(d) An initial and sustained rise in FRP (PA, Fig. 2D).

Mean values of FRP in nine dogs are shown in Fig. 2E (control values).

Mean FRP in CA showed a fall of 10 ms over 12·5 min ($P < 0.05$) followed by a rise to near the control value, patterns (a) and (c) being dominant, whereas mean FRP in PA showed an initial rise at 2·5 min of 15 ms (NS) followed by a progressive fall (patterns (a) and (b) being dominant). On release of the occlusion FRP reverted to normal within 5 min. CA, however, showed an overshoot prolongation of FRP, the mean value reaching a maximum at 7.5 min ($P < 0.02$). This effect did not attain significant levels in PA.

### GRADIENTS OF REFRACTORINESS

Differences or gradients were observed in FRP between each of the three areas studied ΔRP CA-PA, ΔRP NA-CA, ΔRP NA-PA. Following occlusion these RP differences showed a variety of patterns (Fig. 6 control values). Mean values, how-

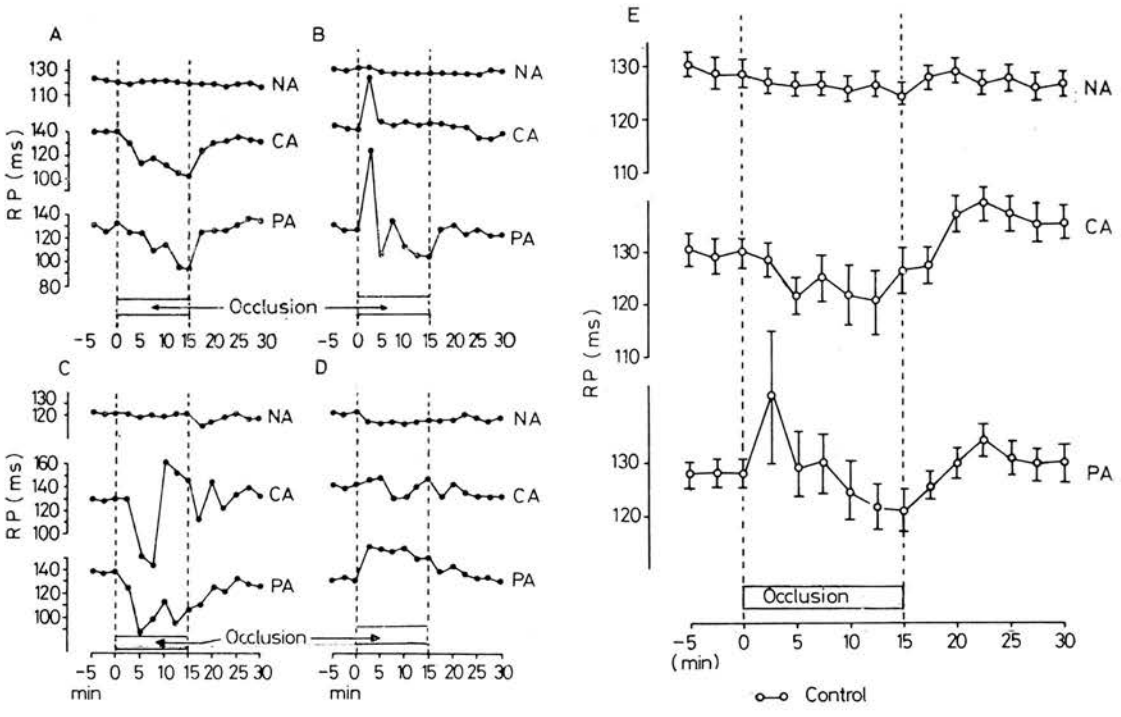


Fig. 2 *Patterns of ventricular refractoriness in normal (NA) and central (CA) and peripheral (PA) ischaemic myocardium during acute coronary occlusion. (A) Top left: shortening of FRP during occlusion. (B) Top right: sharp initial prolongation of FRP followed by shortening during ischaemia. (C) Bottom left: initial shortening followed by prolongation of FRP during occlusion. (D) Bottom right: sustained prolongation of FRP during occlusion. (E) Mean values.*

ever, showed an early rise at 2·5 min of all three gradients ($P < 0.01$) which reverted to control values by 7·5 min. On release a further increase in RP CA-PA was observed in some dogs resulting in a small increase (4 ms) in the mean value at 2·5 min after release. Spontaneous ectopic beats were noted to occur in some dogs at the time of maximum values of RP gradient.

### VENTRICULAR FIBRILLATION

Spontaneous episodes of ventricular fibrillation (VF) occurred in six dogs at various times during occlusion or on release of occlusion. Isoprenaline was not subsequently administered to these dogs.

Two representative studies are shown in Fig. 3A, B. Following coronary occlusion, values of FRP in the two ischaemic zones diverged rapidly, CA (the central ischaemic area) and PA (the marginal ischaemic zone). ΔRP CA-PA was at a maximum value as recorded just before VF for spontaneous fibrillation (Fig. 3A) and just before release for reperfusion fibrillation (Fig. 3B).

Mean values of the three RP gradient values for six dogs are shown in Fig. 3B taken before VF and at 2·5 min intervals preceding VF. A significant rise ($P < 0.02$) in ΔRP is seen during the 2·5 min immediately before VF.

On release of occlusion (Fig. 3D) values of RP immediately preceding release were significantly ($P < 0.005$) greater than when it did not.

### EFFECT OF ISOPRENALINE INFUSION

Isoprenaline 0.1 $\mu g \cdot kg \cdot min^{-1}$ infusion at a constant heart rate (200 $beats \cdot min^{-1}$) equal to that during the first occlusion, produced a significant fall in mean FRP ($P < 0.02$) of 8 ms in the non-ischaemic zone. Similarly falls were observed in CA and PA (5 ms, 6 ms respectively).

Following coronary occlusion similar but more rapid directional changes in FRP occurred as in the control occlusions. A representative case is shown in Fig. 4. A more rapid shortening in FRP after occlusion is seen during isoprenaline infusion in both ischaemic area. This is followed by an

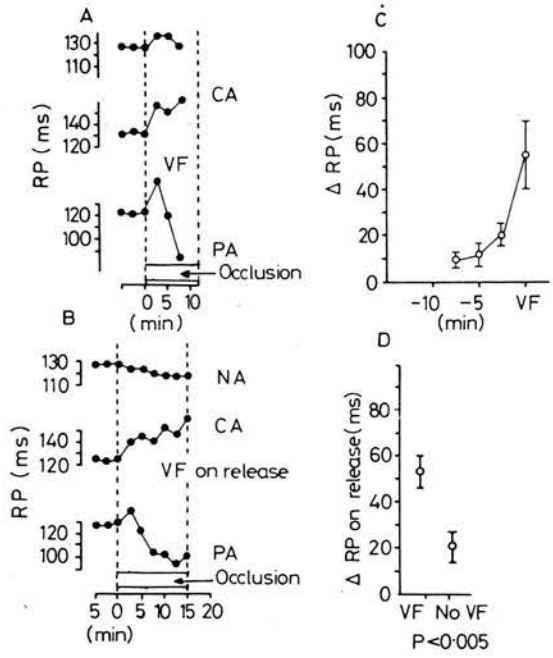


Fig. 3 *(A, C) Above: patterns of refractoriness associated with spontaneous ventricular fibrillation during coronary occlusion (left) in one dog and differences in FRP (ΔRP) between the 3 areas studied, before spontaneous fibrillation in 6 dogs showing rapid divergence of refractoriness in adjacent areas of myocardium (right). (B, D) Below: patterns of refractoriness during coronary occlusion before reperfusion fibrillation in one dog (left) and mean differences in FRP (ΔRP) between the 3 areas studied before release, showing significantly higher values preceding reperfusion fibrillation (right).*

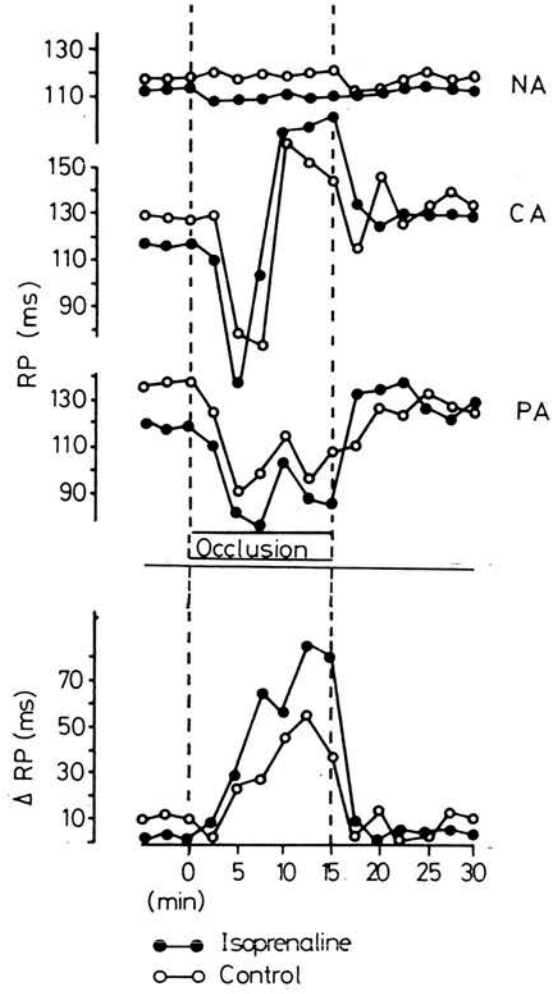


Fig. 4 *Changes in refractoriness in normal (NA), and central (CA) and peripheral (PA) ischaemic myocardium during a 15 min occlusion. A control occlusion (open circles) and an occlusion during infusion of isoprenaline 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ are shown. The difference in FRP (ΔRP) between the two adjacent ischaemic areas is increased during isoprenaline infusion.*

earlier lengthening of FRP in the central area compared with control at a time when FRP in the peripheral area is still falling. This situation of divergence of adjacent FRP leads to an increase in RP gradient (ΔRP).

Mean values of FRP from nine dogs are shown in Fig. 5. They remained significantly less than control ($P < 0.02$) in the non-ischaemic zone during occlusion. Although mean values increased between 5 and 12·5 min with isoprenaline compared with control, they did not differ significantly. In the border area FRP was significantly shortened at 2·5 min ($P < 0.02$).

Mean values of RP gradients are shown in Fig. 6.

With isoprenaline the gradient RP CA-PA was significantly greater than control measurements ($P < 0.05$) at 7·5 min and also 15 min after occlusion ($P < 0.05$). RP NA-PA was significantly increased ($P < 0.05$) at 7·5 min. No significant difference, however, was found for RP NA-CA between normal myocardium and the central ischaemic zone.

On release, FRP followed values of the control run, again with a significantly lower value ($P < 0.02$) in the NA and a small reduction in the central area. This difference was not observed in the peripheral area.

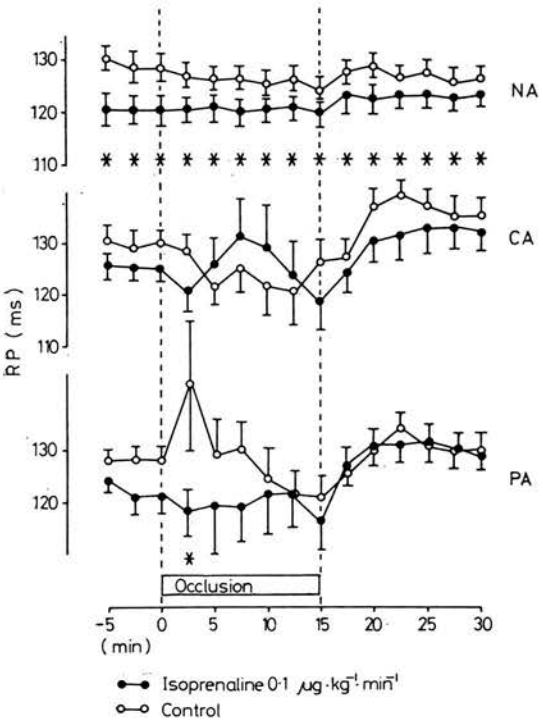


Fig. 5 *Mean changes in refractoriness in normal (NA) and adjacent ischaemic (CA, PA) myocardium during and after acute coronary occlusion in 9 dogs. Open circles represent control measurements and closed circles measurements during isoprenaline infusion 0.1 μg·kg⁻¹·min⁻¹ at the same heart rate.*

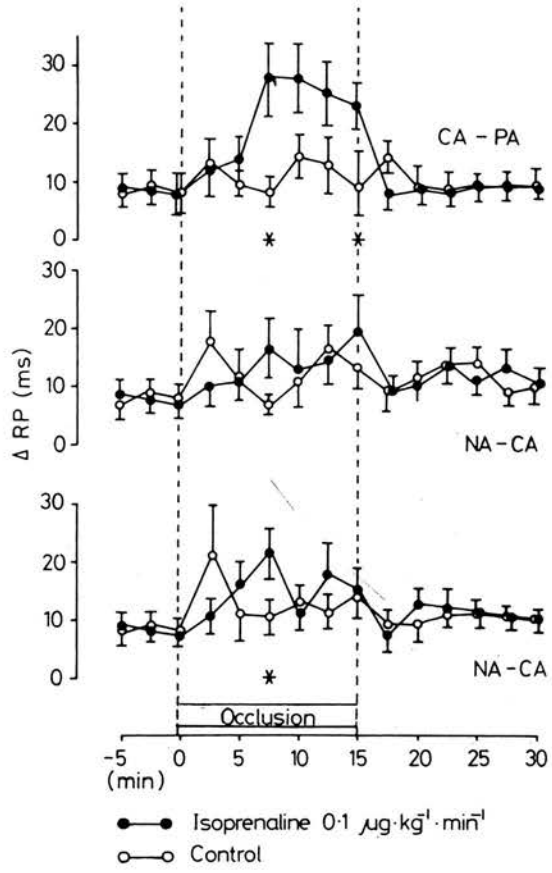


Fig. 6 *Changes in gradients of refractory period (ΔRP) between normal and ischaemic myocardium (NA-CA, NA-PA) and across ischaemic myocardium (CA-PA) during and after acute coronary occlusion. Data as in Fig. 5.*

## Discussion

Acute myocardial ischaemia results in complex electrophysiological changes which may initiate ventricular arrhythmias (Harris, 1950; Scherlag *et al.*, 1970). An important mechanism involved is believed to be that of activation of ventricular myocardium through re-entrant pathways of conduction (Schmitt and Erlanger, 1929; Wit *et al.*, 1974). Slowing of conduction and inhomogeneities of refractoriness which predispose to this process have been demonstrated in ischaemic myocardium (Han, 1969; Scherlag *et al.*, 1974). Disparities in refractoriness of as little as 11 ms in adjacent areas are sufficient to generate local conduction block and delayed conduction of the type associated with re-entrant activity (Allesie *et al.*, 1976).

Our study demonstrates that there are a variety of patterns of change of refractoriness during acute ischaemia. Others have reported apparently contradictory findings describing either reductions (Levites *et al.*, 1975), reductions followed by prolongations (Han, 1972; Elharrar *et al.*, 1977) or prolongations alone of refractory period (Szekeres and Papp, 1971). Our findings show that all of these patterns exist in the first few minutes of acute ischaemia and different patterns may co-exist in central and border areas of ischaemia. The use of occlusion of the diagonal branch of the left anterior descending coronary artery rather than that of the main vessel reduced the possibility of marked conduction delays from the septal myocardium and permitted comparison of change in local refractoriness between normal, border zone and central areas of ischaemia. Thus, a temporal gradient of refractoriness is produced between central and

border ischaemic zones which varies in magnitude according to the duration of ischaemia, the recording sites and the individual dog. Such a gradient is likely to be of importance *per se* in the initiation of arrhythmias. Focal cooling of the epicardium results in more marked prolongation of refractoriness than conduction delay with respect to normal adjacent tissue and is associated with ventricular tachycardia or fibrillation (Wallace and Mignone, 1966). Increases in the local dispersion of refractoriness with myocardial ischaemia accompany a fall in ventricular fibrillation threshold (Han, 1964). In those dogs developing ventricular fibrillation within the 15 min occlusion period marked divergence of change of refractoriness was observed between recording sites and this was significant in the 2·5 min preceding fibrillation. Differences of up to 110 ms were recorded and are of an order to permit the asynchrony of ventricular activation required for fibrillation.

These divergent patterns of refractoriness in ischaemic myocardium may be related to underlying metabolic factors. Refractoriness under normal conditions generally parallels the duration of the cardiac action potential (Hoffman and Cranefield, 1960) but increases in the diastolic excitability threshold during ischaemia dissociate these two variables and prolong refractoriness (Elharrar *et al.*, 1977). Increases in threshold can be produced by elevation of extracellular potassium concentration in the dog (Elharrar *et al.*, 1977) or by superfusion of isolated ventricular muscle with ischaemic venous effluent blood (Downar *et al.*, 1977), this latter effect being attributable not to potassium but some ischaemic myocardial depressant factor. The initial shortening of refractoriness following occlusion (Fig. 1A, C) would be explicable on the basis of a shortened action potential. Such shortening has been demonstrated both *in vivo* (Prinzmetal *et al.*, 1968) and *in vitro* (Trautwein, 1954) and is related to metabolic dependency on the slow inward calcium flux (McDonald and MacLeod, 1973). The phenomenon of post-repolarisation refractoriness (Lazzara *et al.*, 1975) may contribute to the subsequent prolongation, possibly as a result of delayed recovery from inactivation of the rapid inward current (Gettes and Reuter, 1974).

The arrhythmogenic effect of catecholamines or sympathetic stimulation is well described (Moore *et al.*, 1964; Ceremuzynski *et al.*, 1969) as is their effect on reduction of ventricular fibrillation threshold and increased local dispersion of refractoriness during myocardial ischaemia (Han *et al.*, 1964). The normal refractory period was shortened in our series and the directional changes during ischaemia enhanced in keeping with the known effects of sympathetic stimulation (Tsien *et al.*, 1972; Han *et al.*, 1964). The increased gradient of refractoriness between border and central ischaemic area suggests that not only are local inhomogeneities increased but that large differences are generated by catecholamines between distantly separated areas of myocardium. It is of interest that maximum dispersion occurred after 5 min of ischaemia, a time at which the ventricular fibrillation threshold is at a minimum (Lown and Verrier, 1976). Rate-dependent effects were eliminated by overdrive pacing. *In vivo*, however, catecholamine induced rate-dependent changes may in themselves influence refractoriness. Although a slowing of heart rate *per se* increases dispersion of refractoriness and decreases fibrillation threshold (Han, 1969) during ischaemia an increased rate augments the degree of ischaemia with similar effect (Kent *et al.*, 1973). The contribution of changes in refractoriness to catecholamine induced arrhythmias must however be weighed against their effects on enhancement of ventricular automaticity and induction of slowly conducting 'slow potentials' (Cranefield and Hoffman, 1971).

The phenomenon of reperfusion fibrillation has been well described experimentally (Sewell *et al.*, 1955; Battle *et al.*, 1974) and has been related to the sudden washout of metabolites such as potassium or lactate (Lang *et al.*, 1974). Our finding of a greater disparity of refractoriness between central and border ischaemic zones before release in those dogs developing reperfusion fibrillation would suggest a further correlate with severity of pre-existing electrophysiological abnormality.

The initiation of ventricular fibrillation in the early phase of acute myocardial ischaemia has been variously ascribed to abnormal automaticity or re-entrant activity in both muscle and conducting tissue (Bigger *et al.*, 1977). The relative importance of dispersion of refractoriness as opposed to slowed conduction or intrinsic pacemaker activity is unclear. This study supports the concept of a relationship between increased disparities of refractoriness across the ischaemic myocardium and arrhythmogenicity under basal and catecholamine-stimulated conditions. It is likely therefore that therapeutic interventions directed towards reducing these disparities may reduce the incidence of ventricular fibrillation.

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